

OTTAWA RIVER REPORT

by

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OTTAWA RIVER REPORT - 1970

PART I

SOME ECOLOGICAL ASPECTS OF SULFATE-REDUCING AND
SULFUR OXIDIZING BACTERIA IN AREAS OF THE OTTAWA
RIVER AFFECTED BY PULP AND PAPER MILL WASTES.

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PART I - SOME ECOLOGICAL ASPECTS OF SULFATE-REDUCING
AND SULFUR-OXIDIZING BACTERIA IN AREAS OF THE
OTTAWA RIVER AFFECTED BY PULP AND PAPER MILL
WASTES.

INTRODUCTION AND REVIEW OF THE LITERATURE

Bacteriological parameters, e.g. fecal coliforms and fecal streptococci associated with sewage and domestic wastes serve an important function in determining water quality from the public health standpoint (2). With the increasing degree of water pollution by industry, it is becoming more important to understand the ecological implications of industrial pollutants with all life forms, including the microbiota of receiving waters. Therefore, knowledge of aquatic microbial ecology may serve a purpose to indicate changes in the environment brought about by the discharged wastes. A particular pollutant may be detrimental to the growth of a given microorganism (if toxic) or may be beneficial to the organism if it serves as a growth nutrient. Changes in natural bacterial populations in a watercourse receiving wastes from industry may have a potential application in determining the source and extent of pollution.

Since wastes from pulp and paper mills contain appreciable quantities of organic and inorganic sulfur compounds (4,5,9,23), a search for bacteria which use sulfur compounds for growth might be advantageous in indicating river pollution of this nature.

For this reason, a survey was conducted on the Ottawa River to determine the natural populations of sulfate-reducing and sulfur-oxidizing bacteria in the water and sediments at selected stations above and below the outfalls from pulp and paper mills.

The role of various physiological groups of microorganisms in the degradation of components of pulp and paper mill wastes in the rivers receiving them has been dealt with in only a few cases. Luchterowa (10) evaluated water pollution by the occurrence of physiologically different groups of bacteria and suggested that the more polluted areas of a river contained higher bacterial populations with a greater diversity and intensity of metabolic processes. Paluch (12) investigated seasonal changes in the microflora of the Mleczna River (Poland) following discharges of pulp and paper mill effluents. He observed that the river microflora below the effluent was qualitatively similar to that of the pulp mill waste waters. In river water, total counts decreased in winter and increased towards the end of summer, with counts ranging from 10^5 - 10^{10} per 100 ml. Below the discharge, nitrifying bacteria were found in June only, while denitrifiers, cellulose decomposers and sulfur bacteria appeared in low numbers at all seasons.

The sulfur bacteria are a heterogeneous group of microorganisms which have in common, the characteristic that inorganic sulfur compounds play a major role in their metabolism, and hence

these bacteria are important to the sulfur cycle in nature. The autotrophic sulfur-oxidizing bacteria of the genus Thiobacillus derive their energy from the oxidation of reduced inorganic sulfur compounds, e.g. sulfur, sulfide, thiosulfate. The thio-bacilli are aerobic or microaerophilic and are able to use CO_2 as their sole carbon source for growth. The most common species are Thiobacillus thiooxidans, an acidophilic autotroph with an optimum pH of 3.0 and T. thioparus, which shows optimum growth at a neutral pH. Several review articles have treated the subject of sulfur metabolism by these specialized bacteria in the environment (6,18,22).

Sulfate-reducing bacteria are anaerobic organisms which obtain their energy by dissimilatory reduction of sulfate to hydrogen sulfide, while growing on certain organic compounds, e.g. organic acids, amino acids, alcohols and simple sugars. The sulfate serves as the oxidizing agent for the organic compounds which are sources of energy for growth. The most common species, Desulfovibrio desulfuricans is widespread in nature and develops best at a pH range of 5.5 - 9.0 at strongly reducing conditions ($\text{Eh} < -200$ mv). In pure culture, Desulfovibrio apparently was able to grow on a pyruvate medium devoid of sulfate (8,11). In natural conditions however, it was reported that these bacteria which were not usually a major group in the anaerobic digestion of raw sewage sludge, increased in number to high levels when

sulfates were added (20). A more detailed treatment of the physiology and economic importance of sulfate-reducing bacteria is given in several review articles (13,15,18,19).

Pulp and paper mill wastewaters contain substances that promote the growth of sulfate-reducers in the receiving waters and river sediments, resulting in the production of toxic H_2S which may impair water quality. The biodegradation of cellulose produces simple sugars and organic acids which serve as organic substrates for growth of Desulfovibrio. Depletion of dissolved oxygen in water below outfalls from paper mills may be caused by microbial attack of decomposable organic matter and by the oxidation of reduced inorganic sulfur to sulfate by the sulfur-oxidizing bacteria. Conditions of reduced D.O. are conducive to the growth of sulfate-reducing bacteria (19).

Hata et al. (7) have studied the significance of pulp mill wastes on the production of hydrogen sulfide in coastal waters affected by these wastes. These workers found fluctuations in the abundance of sulfate-reducing bacteria in the sediments. Populations of Desulfovibrio sp. and concentrations of sulfide and organic matter increased with proximity to the pulp and paper mill. Numbers of sulfate reducers and sulfide concentration were directly correlated with the organic content of the sediment suggesting that Desulfovibrio sp. were mainly responsible for the production of H_2S in the sediments. In certain habitats, sulfide

production was suppressed primarily by the scarcity of sulfate, in others by the paucity of organic matter. Large quantities of organic matter brought onto the bottom mud by sewage or industrial wastes stimulated the growth and physiological activity of the sulfate-reducing bacteria.

Methods:

The bacteriological survey concentrating on sulfur bacteria was carried out in co-operation with Water Quality Surveys Branch during the summer of 1969. The survey was concentrated on three reaches of the Ottawa River affected by effluents from pulp and paper mills. A section of the river in the Temiskaming-Mattawa area was surveyed July 21-26 only. Three separate surveys from June 16-20, August 19-22 and September 22-25 were conducted on the Ottawa River in two reaches from Ottawa-Thurso and from l'Orignal-Carillon. Sediment and water samples were refrigerated and analyzed within 24 hours of the time of sampling for sulfate-reducers (Desulfovibrio sp.), and sulfur oxidizers (Thiobacillus thioparus, T. thiooxidans). Determination of total plate count (20°C) and nitrifying bacteria was carried out on certain water samples.

A membrane filtration method (MF) was used for the enumeration of sulfate-reducing bacteria (Desulfovibrio sp.) in water and sediments. This technique was essentially a modification of the submerged membrane plate method described by Tsuneishi and Goetz (21). The medium used for the MF count was based on a modification of the media described by Postgate (14) and Tsuneishi and Goetz (21). The Desulfovibrio medium consisted of: K_2HPO_4 , 0.6 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; NH_4Cl , 1.0 g; $CaCl_2$, 0.05 g; Na_2SO_4 , 1.0 g; sodium lactate, 10.0 g; yeast extract, 1.5 g; $Fe(NH_4)_2(SO_4)_2$, 0.1 g; sodium thioglycollate, 0.3 g; agar, 10.0 g; distilled water, 1000 ml; pH 7.2. Stock solutions of ferrous ammonium sulfate (1%) and sodium thioglycollate (1.5%) previously sterilized by membrane filtration were added (1 ml per 100 ml) to each bottle of molten agar just before pouring the plates. A thin layer of the agar medium for sulfate-reducers was poured into small "Millipore" petri dishes and allowed to set. After filtration of the sample, the appropriate membrane was placed with its surface facing downward on the agar. A thick layer of the agar medium was poured over the inverted membrane to a level near the upper rim of the plate. The plates were incubated anaerobically at 25°C for 10 days in Brewer jars using the H_2 - CO_2 "Gas-Pak" system (Baltimore Biol. Labs.). Growth of sulfate-reducing bacteria was indicated by the formation of (black) Fe S in the environment of each colony. The development of colonies was very slow and sometimes re-incubation of the plates

for an additional week was necessary. Many colonies appeared as small black spots (< 1 mm diameter) on the membrane. In some cases, intense dark zones spread across much of the plate area. Subsequent investigations in the laboratory showed that if the membrane was placed upright on the plate and sandwiched between equal agar layers, the dark zone spreading was much reduced, while colony development appeared the same, as when the membrane was inverted.

Bacteria picked from the black colonies were anaerobic, gram negative, motile, curved rods which showed growth and sulfate reduction in Starkey's broth (17), and were thus characteristic of the genus Desulfovibrio.

A most probable number method (MPN) was employed for the enumeration of chemoautotrophic sulfur oxidizing bacteria. Thio-sulfate broth media used for the cultivation of thiobacteria were described by Postgate (16) for Thiobacillus thio-parus and T. thio-oxidans.

In the MPN procedure, a 3 test tube series of each sterile medium was inoculated with aliquots of each sample or dilutions thereof. All tubes including sterile controls were incubated in the dark for 4 weeks at room temperature (20° - 25° C). At the end of the incubation period, all MPN tubes were checked for the presence or absence of bacterial growth. Determination of the growth of the thiobacilli was made by testing the pH and sulfate concentration of the contents of the incubated test tubes and

comparing the results with those determined for sterile control media. A slight white precipitate presumably due to chemical production of elemental sulfur sometimes formed in sterile T. thiooxidans broth, but pH was unchanged. In certain inoculated tubes a cream colored precipitate of elemental sulfur appeared along with a depression of pH.

A drop of thiosulfate broth from each tube in the MPN series was placed on a small segment of pH indicator paper (pH range 2-10). If the pH of the broth in the test tube had dropped appreciably from that of the sterile control tubes, a positive reading was designated for acid production in that tube. An approximate determination for sulfate production in each tube of thiosulfate broth was made by adding 1 ml of the test broth to a large test tube containing 18 ml distilled water. One ml of a 2% BaCl_2 solution was then added to each large tube and the contents shaken. Control tubes of sterile T. thiooxidans and T. thioparus media were treated in the same way. Turbidity in each tube after BaCl_2 addition could readily be measured by a visual comparison with the control. A definite milky turbidity of BaSO_4 appeared in positive tubes which was discernibly greater than the faint haze which developed in the control and negative tubes. Estimation of the numbers of thiobacilli per 100 ml was made by recording the number of positive tests and determining the count from a standard MPN table.

Results and Discussion:

(1) Temiskaming Survey

A map of the section of the Ottawa River surveyed in the Temiskaming-Mattawa region is illustrated in Figure 1. A description of the sampling stations for each range is given in Table I, and the individual bacterial counts obtained at each station are presented in Tables I and II in the Appendix.

The distribution of bacteria in surface water is illustrated graphically for stations near the Quebec shore (Fig. 2) and Ontario side (Fig. 3) of the river. Numbers of sulfate-reducers (Desulfovibrio) increased from 20/100 ml at station 1 (Temiskaming dam above the mill) to 7.0×10^3 /100 ml at A-2 below the mill (Fig. 2). A degree of increase in numbers of sulfur-oxidizers (Thiobacillus thioparus) similar to that observed for sulfate-reducers was found between stations 1 (< 3 /100 ml) and A-2 (2.0×10^3 /100 ml). Numbers of sulfur bacteria at station 2, in the effluent from the CIP (Temiskaming) mill, were lower than those found in river water at ranges A, B and C, below the discharge point. This observation was especially true for the sulfur-oxidizers which were found in low numbers (9/100 ml) in the effluent than for the sulfate-reducers which reached a population of 1.4×10^2 /100 ml in the waste water. This would suggest that multiplication of thiobacilli was occurring in the water below the discharge of the mill effluent. The concentrated mill wastes may

Figure 1 Section of the Ottawa River in the Timiskaming-Mattawa area showing ranges of sampling stations and location of CIP mill (Timiskaming).



TABLE I - Description of ranges and sampling stations on
Ottawa River (Temiskaming-Mattawa).

Station	Location*	Mileage (from mouth)
1	Control - Ontario side below Temiskaming dam	372.5
2	CIP (Temiskaming) - mill effluent	
A-2	Thorne (below mill effluent Quebec side)	371.9
A-3	Thorne (1/2 way across river from Quebec side)	
A-5	Thorne (3/4 way across river from Quebec side)	
B-6	1/4 way across river from Quebec side	370.8
B-8	3/4 way across river from Quebec side	
C-9	1/4 way from Quebec side	369.3
C-10	1/2 way from Quebec side	
D-12	1/4 way from Quebec side	366.4
D-14	3/4 way from Quebec side	
E-15	1/3 way from Quebec side	363.8
F-19	1/2 way from Quebec side	362.3
F-20	3/4 way from Quebec side	
G-21	1/3 way from Quebec side	358.7
H-24	1/3 way from Quebec side (below Coxie's Camp)	355.7
I-27	1/3 way from Quebec side	353.1
J-30	1/2 way from Quebec side	349.8
K-33	1/3 way from Quebec side	347.6
L-37	1/2 way from Quebec side (Wilson's Landing)	345.1
M-40	1/2 way from Quebec side (above Otto Holden)	341.3
M-41	3/4 way from Quebec side (above Otto Holden)	
N-43	1/2 way from Quebec side (below Otto Holden and above Mattawa)	339.7
MR-1	Mattawa River -11/2 mi upstream from mouth	
U -64	1/2 way from Quebec side - 1/4 mi below Deux Rivieres	313.6
Z -79	1/2 way from Quebec side (Stonecliffe)	296.3

* ranges marked by hydrographic buoys or markers on shore.

LOG COUNT PER 100 ml

PARAMETERS

- × Total Plate Count
- Sulfate-Reducers
- ▲ *Thiobacillus thioparus*

mill effluent

1 2 A B C D E F G H I J K L M N O P Q R S T U V W X Y Z

SAMPLING RANGES DOWN RIVER

Figure 2 - Bacterial distribution in surface water at stations near Quebec side of Ottawa River below Temiskaming (July, 1969).

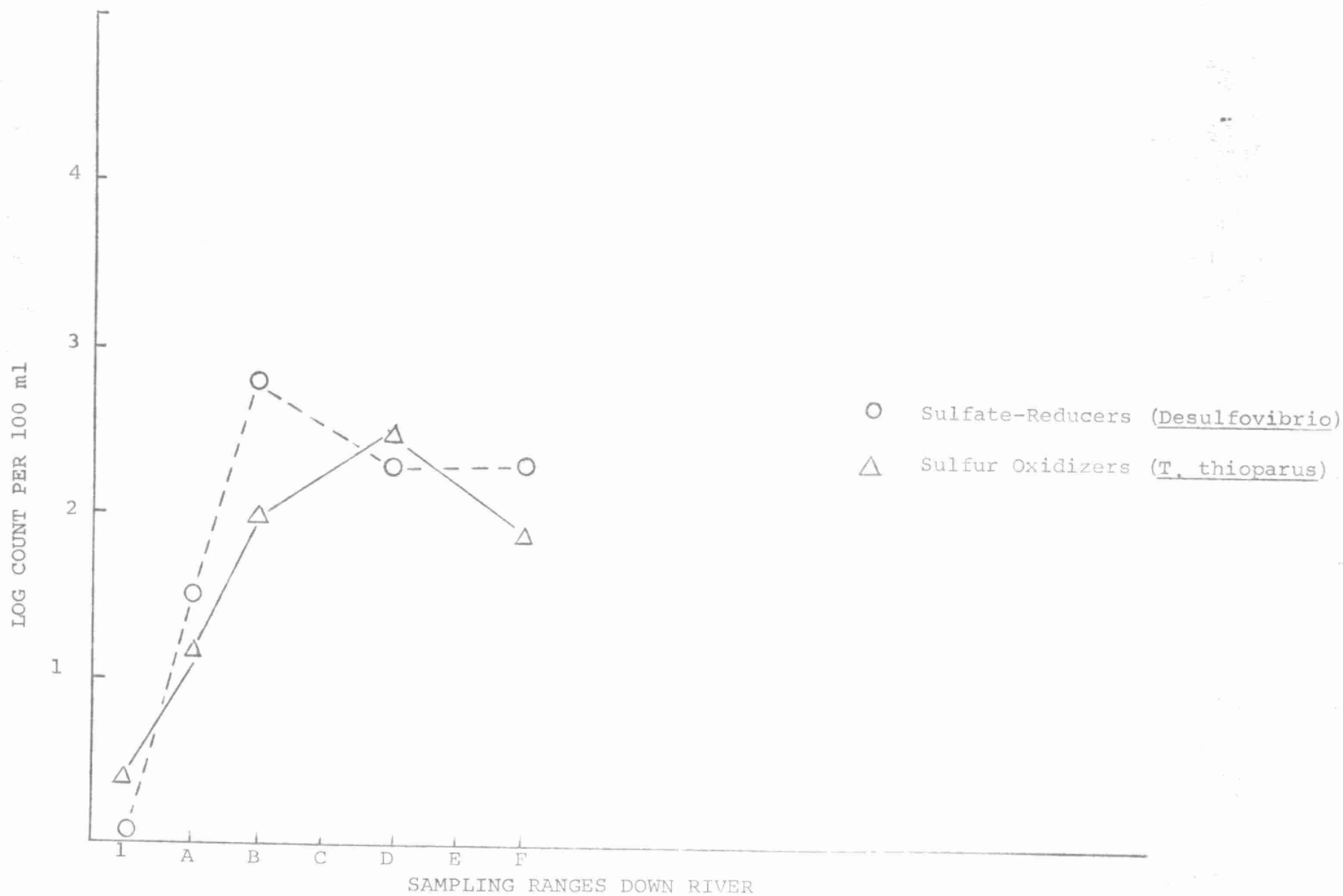


Figure 3 - Bacterial distribution in surface water at stations near Ontario side of Ottawa River below Temiskaming, July, 1969.

have been detrimental to growth of the thiobacilli by virtue of their low Eh or presence of toxic agents. Increasing dilution of the wastes in the oxygenated river water below the mill outfall may have been favorable for growth of the sulfur-oxidizers on inorganic sulfur compounds, e.g. sulfide, thiosulfate, elemental sulfur, present in the inorganic form originally or liberated microbiologically from organic sulfur components of the waste liquors.

The presence of reducing agent, e.g. thiosulfate, sulfide, sulphydryl-containing organics in the effluent which would be conducive to growth of sulfate-reducers may have accounted for the appreciable population of Desulfovibrio in the mill waste. At range A below the outfall, the count of sulfate-reducing bacteria in water was determined as $7.0 \times 10^3/100$ ml. The population declined to approximately $3.0 \times 10^2/100$ ml at ranges B, D and F. Since the counts at stations A, B, D and F were as great or greater than those observed for the raw effluent, and considering the dilution factor of the river water, it appeared that some bacterial multiplication was occurring in the river below the outfall. It is possible that growth of Desulfovibrio in zones of the river below the discharge point may have occurred in the interstices of particulate waste organics suspended in the water. Within this microhabitat replete with nutrients and protected from the external aerobic environment by the presence and abundance of

reducing agents and organic matter, Desulfovibrio cells conceivably may have multiplied.

The peak population of Thiobacillus thioparus which occurred at ranges A and B ($2.0 \times 10^3/100$ ml) declined gradually at stations below range B, reaching low values $< 10/100$ ml at range L and below. Although a slight increase in population of 40/100 ml occurred at range M just above the Otto Holden dam, no significance can be attached to this variation because the results are based on the data obtained at one sampling time only. Counts of Desulfovibrio closely paralleled counts of Thiobacillus at stations down river. Although a slight increase in numbers of sulfate-reducers occurred at stations M and U of 10 and 15 cells per 100 ml, the bacterial population in the lower reaches of this section of the river was considerably less than the bacterial density observed in proximity to the Temiskaming mill. Depletion or scarcity of available sulfur substrates in the water may have been responsible for the lower populations of sulfate-reducing and sulfur-oxidizing bacteria observed at ranges below F. Sulfur bacteria were not detected in water at station MR-1 on the Mattawa River which served as a control free of influence by pulp and paper mill wastes.

Although higher values were obtained for total plate counts ($10^3 - 10^5/100$ ml) than for counts of sulfur bacteria, the heterotrophic population in the river from stations A-N was relatively constant as compared to the marked variation in counts of sulfur bacteria. Total counts were not markedly influenced by mill discharges. Total counts therefore did not appear to be as reliable or as sensitive as counts of sulfur bacteria in detecting the pollution source or indicating waters grossly contaminated by paper mill wastes.

At range A, numbers of the sulfur group were considerably less on the Ontario side ($45/100$ ml) than near the Quebec side of the river ($2.0 \times 10^3/100$ ml). However, by range B, D and F, populations of sulfur-oxidizers and sulfate-reducers were generally the same at corresponding Ontario and Quebec stations. This would suggest that waste waters from Temiskaming were mixing with water towards the Ontario shore by these stations or sources of pollution (sewage) from the Village of Thorne on the Ontario side of the river at range B were contributing to the high counts at the Ontario stations B, C and D.

Changes in the population of sulfate-reducing and sulfur-oxidizing bacteria in sediments of the Ottawa River below Temiskaming are depicted in Figures 4 and 5 for stations near the Quebec and Ontario sides respectively. At range A, below the CIP

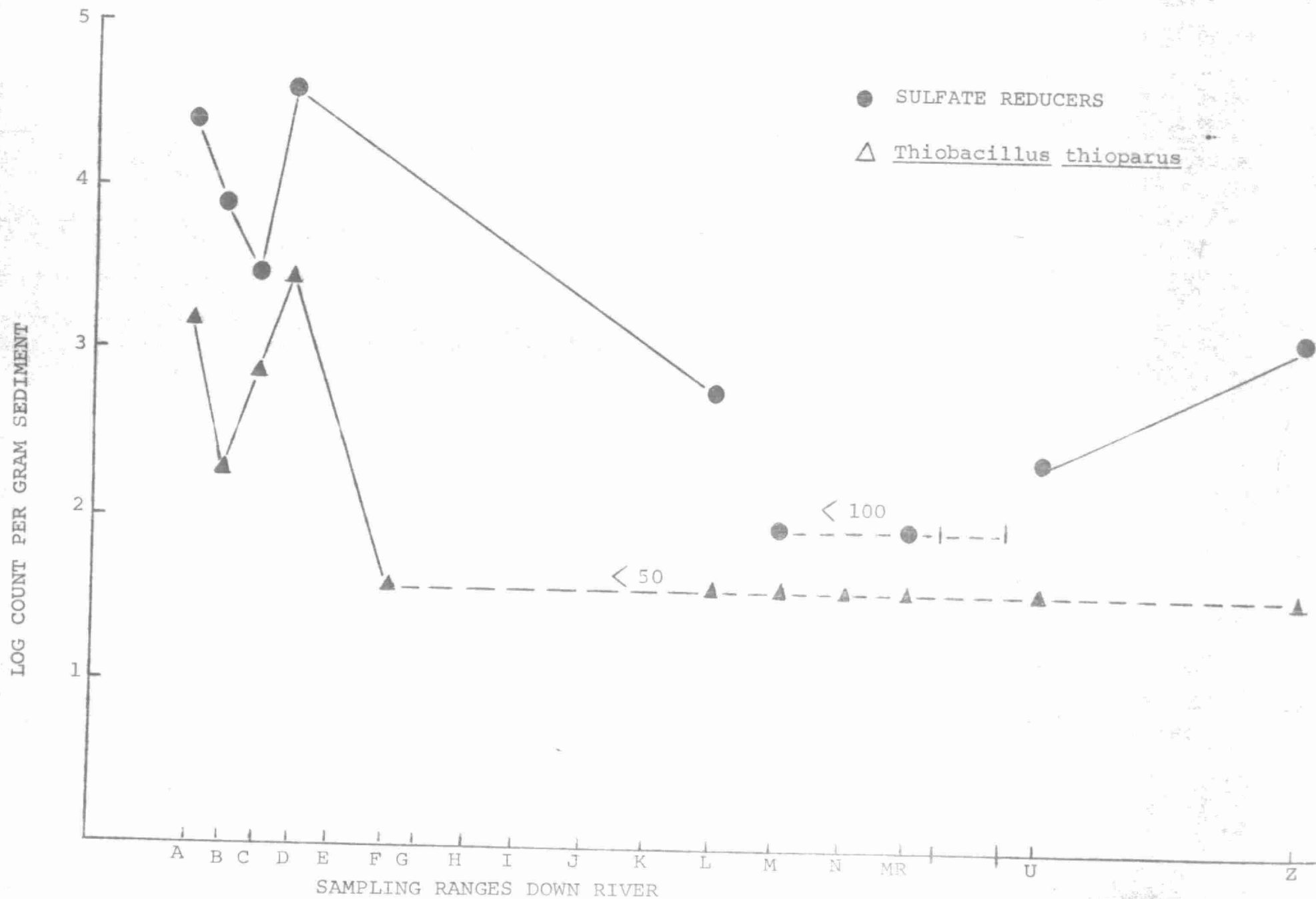


Figure 4 - Counts of sulfate-reducing and sulfur oxidizing bacteria in sediment from the Ottawa River at Quebec stations below Temiskaming (July, 1969).

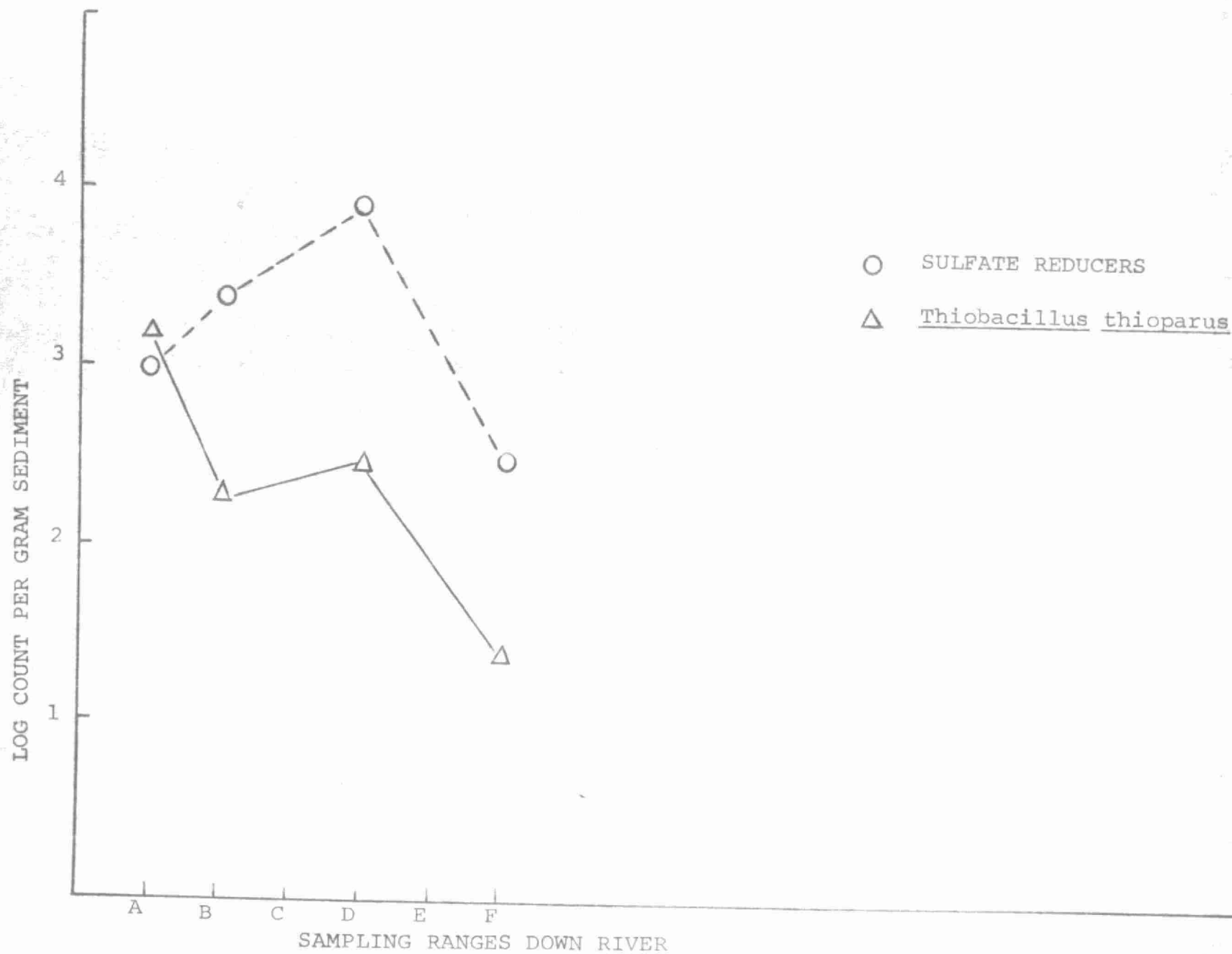


Figure 5 - Counts of sulfate-reducing and sulfur oxidizing bacteria in sediments from the Ottawa River at Ontario stations below Temiskaming (July, 1969).

outfall, the count of Desulfovibrio was $2.2 \times 10^4/\text{g}$ at the Quebec station compared to $1.1 \times 10^3/\text{g}$ on the Ontario side. At stations near the Quebec shore, numbers declined to 8.7×10^3 and $2.9 \times 10^3/\text{g}$ at B and C and increased to $4.0 \times 10^4/\text{g}$ at D. On the Ontario side, the count of sulfate-reducers in the sediment reached a maximum of $7.7 \times 10^3/\text{g}$ at D, decreasing at F to $3.2 \times 10^2/\text{g}$. At Quebec station L, a count of only 5.9×10^2 was recorded for sulfate-reducers, while at M and MR, none were detected ($< 100/\text{g}$). Below Deux Rivieres at U and Z, numbers of Desulfovibrio increased slightly to 2.8×10^2 and 2.0×10^3 per gram sediment. Although counts of sulfur-oxidizers in the sediments were approximately one order of magnitude less than those obtained for sulfate-reducers, a similar trend in population distribution appeared at most stations. Numbers of thiobacilli fluctuated with peaks occurring at ranges A and D. At station F and below, including the Mattawa River station, MR-1, no thiobacilli were detected in sediment samples ($< 50/\text{g}$). Highest counts of sulfate-reducers and sulfur-oxidizers were observed at stations A and D, below the outfall of CIP (Temiskaming). Highest counts of sulfate-reducing bacteria at the ranges immediately below CIP (Temiskaming) would strongly suggest enrichment of these bacteria by sulfate and organic matter contributed by the paper mill wastes. In the sedimentary environment below the mill outfall where sulfur compounds would be abundant, high populations of sulfur-oxidizing bacteria may have

developed, the thiobacilli utilizing reduced inorganic sulfur compounds of the paper mill wastes or the H_2S generated by the sulfate-reducers. The establishment of a dynamic equilibrium between the processes of sulfate reduction and sulfur oxidation occurring concurrently in the sediment would be influenced by a complex interaction of physical, chemical and biological factors in the environment. Of significance in assessing the degree of pollution is the relative number of bacteria present or trend in population distribution in various reaches of a river rather than actual numbers. Although actual numbers of sulfur bacteria were considerably less than total plate counts, the peaks in populations of the former group delineated pollution sources from the CIP (Temiskaming) mill, more clearly than did levels of the total heterotrophic population.

(2) Ottawa-Carillon Survey

Figures 6 and 7 depict the section of the Ottawa River from Ottawa-Carillon which was surveyed in June, August and September of 1969. Descriptions of sampling station locations are presented in Table 2. Appendix Tables III and IV list the counts of various bacterial parameters found in water and sediment during June, August and September. There was very little variation between the bacterial counts obtained at each sampling time. The geometric means of counts of sulfur bacteria obtained at all river stations for each of the three sampling times were determined. No significant

Figure 6 Section of the Ottawa River from Ottawa to Carillon,
showing sampling stations and major outfalls
from pulp and paper mills.

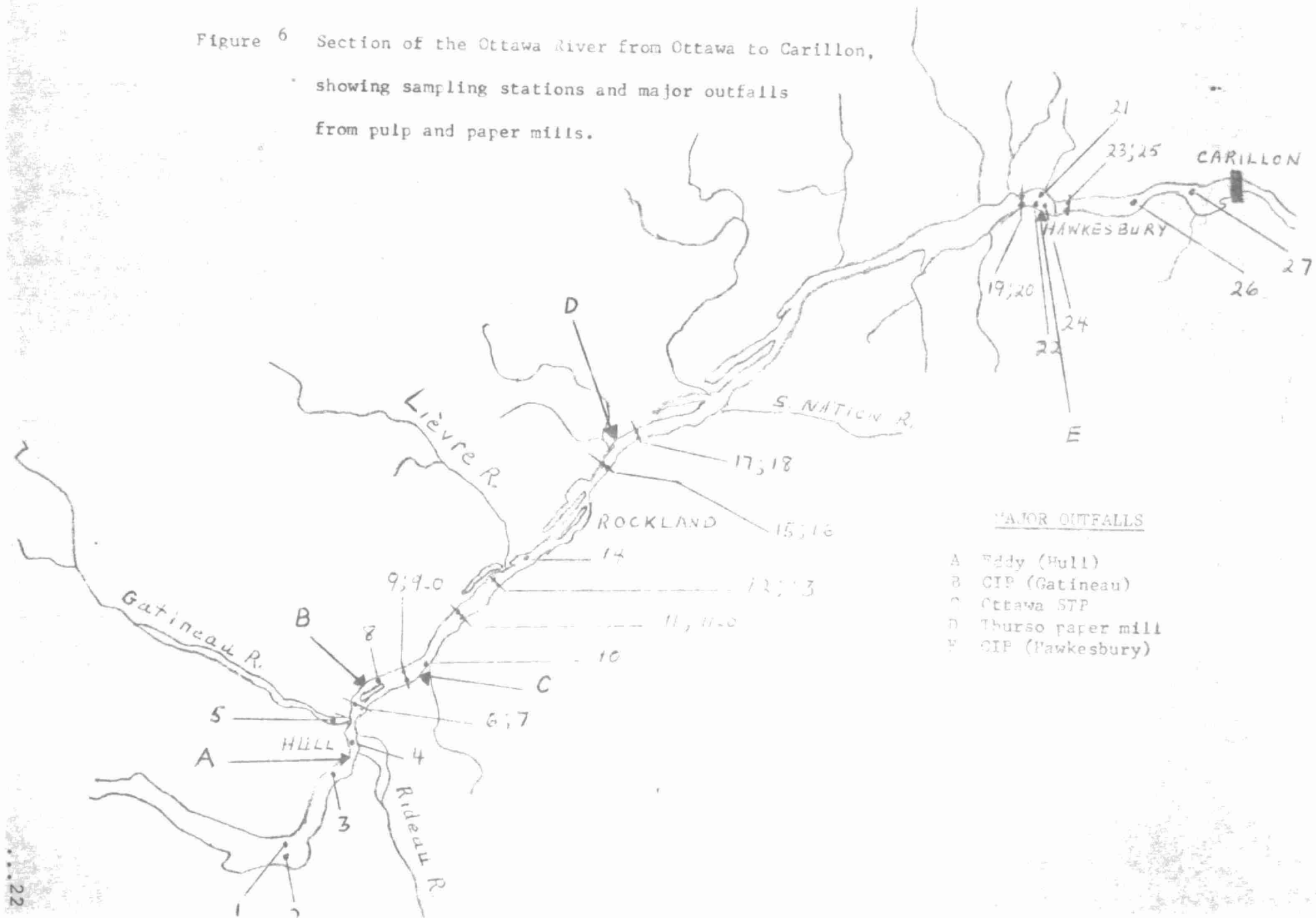




Figure 7 Section of the Ottawa River between Original and Chute-a-Blondeau showing sampling stations and location of the Canadian International paper mill at Hawkesbury.

TABLE II - Description of sampling stations on Ottawa River
(Ottawa-Carillon)

<u>Station</u>	<u>Location Description</u>
1	Lac Deschenes - 1 1/2 mi. above Brittania yacht club (1/3 way from Quebec shore)
2	Lac Deschenes (3/4 way from Quebec shore)
3	1/4 mi. above E.B. Eddy (Hull) (1/3 way from Quebec shore)
4	1/2 mi. below E.B. Eddy (Hull) (1/4 way from Quebec shore)
5	Gatineau River (1/4 mi. above Highway 8 bridge)
6	Kettle Island (1 mi. below Gatineau mouth (1/4 way from Quebec shore)
7	Rockcliffe Park (3/4 way from Quebec shore)
8	Kettle Island (Quebec channel - approx. 1 mile below CIP (Gatineau) discharge)
8.5	CIP (Gatineau) mill effluent
9	Upper Duck Island - 1/4 mi. from Quebec shore
9-0	Junction of Upper and Lower Duck Islands (1/4 mi. from Ontario shore above Ottawa STP outfall)
10	1/2 mi. below Ottawa STP (100 yds. from Ontario shore)
11	1 1/2 mi. below Hiawatha Park (1/3 way from Quebec shore)
11-0	1 1/2 mi. below Hiawatha Park (2/3 way from Quebec shore)
12	1/2 mi. above mouth Lievre River (1/4 way from Quebec shore)
13	1/2 mi. above mouth Lievre River (3/4 way from Quebec shore)

TABLE II - continued

<u>Station</u>	<u>Location Description</u>
14	1 mi. below mouth Lievre River (1/4 way from Quebec shore)
15	Rockland (1 mi. above Thurso paper mill) - 1/4 way from Quebec shore
16	Rockland (1 mi. above Thurso paper mill) - 3/4 way from Quebec shore
15.5	at lagoon effluent from Thurso mill
17	1 mi. below Thurso mill (1/4 way across river from Quebec)
18	1 mi. below Thurso mill (3/4 way across river from Quebec)
19	2 mi. below l'Orignal (1/4 way from Quebec shore)
20	2 mi. below l'Orignal (3/4 way from Quebec shore)
21	Grenville (1/4 way from Quebec shore)
22	at CIP (Hawkesbury) - 100 yd. west of Hamilton Island
22.5	at CIP (Hawkesbury) mill effluent discharge - south of Hamilton Island
23	1/4 mi. below Perley bridge (1/4 way from Quebec shore)
24	East of Hamilton Island (between island and bridge) (1/2 mi. below discharge from mill)
25	1/2 mi. below Perley bridge - approx. 200' from Ontario shore
26	above Chute à Blondeau (1/2 way across river - Greece's Point)
27	1 mi. below Chute à Blondeau (1/2 way across river).

difference was found between the geometric means for a specific bacterial count for June, August and September (χ^2 test; 95% level). The geometric mean, representing the average count for each station for the summer was calculated and the results plotted graphically to portray the mean bacterial distribution at each station for the season.

The mean distribution of sulfate-reducers and thiobacilli in surface water at the sampling stations on the Ottawa River between Ottawa and Thurso are depicted in Figures 8 and 9 (summer, 1964).

Generally speaking, bacterial counts were lower at stations near the Ontario side than at the corresponding Quebec stations. Counts of sulfate-reducers and sulfur-oxidizers were lowest at stations 1, 2 (Lac Deschenes) and 5 (Gatineau River) ($<2-10/100$ ml). Numbers of Desulfovibrio increased to $8.0 \times 10^2/100$ ml at station 4 below Eddy (Hull), and to $3.0 \times 10^3/100$ ml at the effluent from CIP (Gatineau) (8.5), then declined at stations 8 and 9 to 8.0×10^2 and 1.6×10^2 per 100 ml, respectively. The population remained relatively constant in the low hundreds per 100 ml at Quebec stations down river from Upper Duck Island (station 9) to below Rockland (station 15). A slight increase in numbers of sulfate-reducers to $8.0 \times 10^2/100$ ml occurred at station 15.5 below the Thurso paper mill discharge. Numbers of sulfate-reducers remained fairly stable at levels between 10-100/100 ml at stations near the Ontario shore from 7 (Rockcliffe Park) to 18 (Thurso).

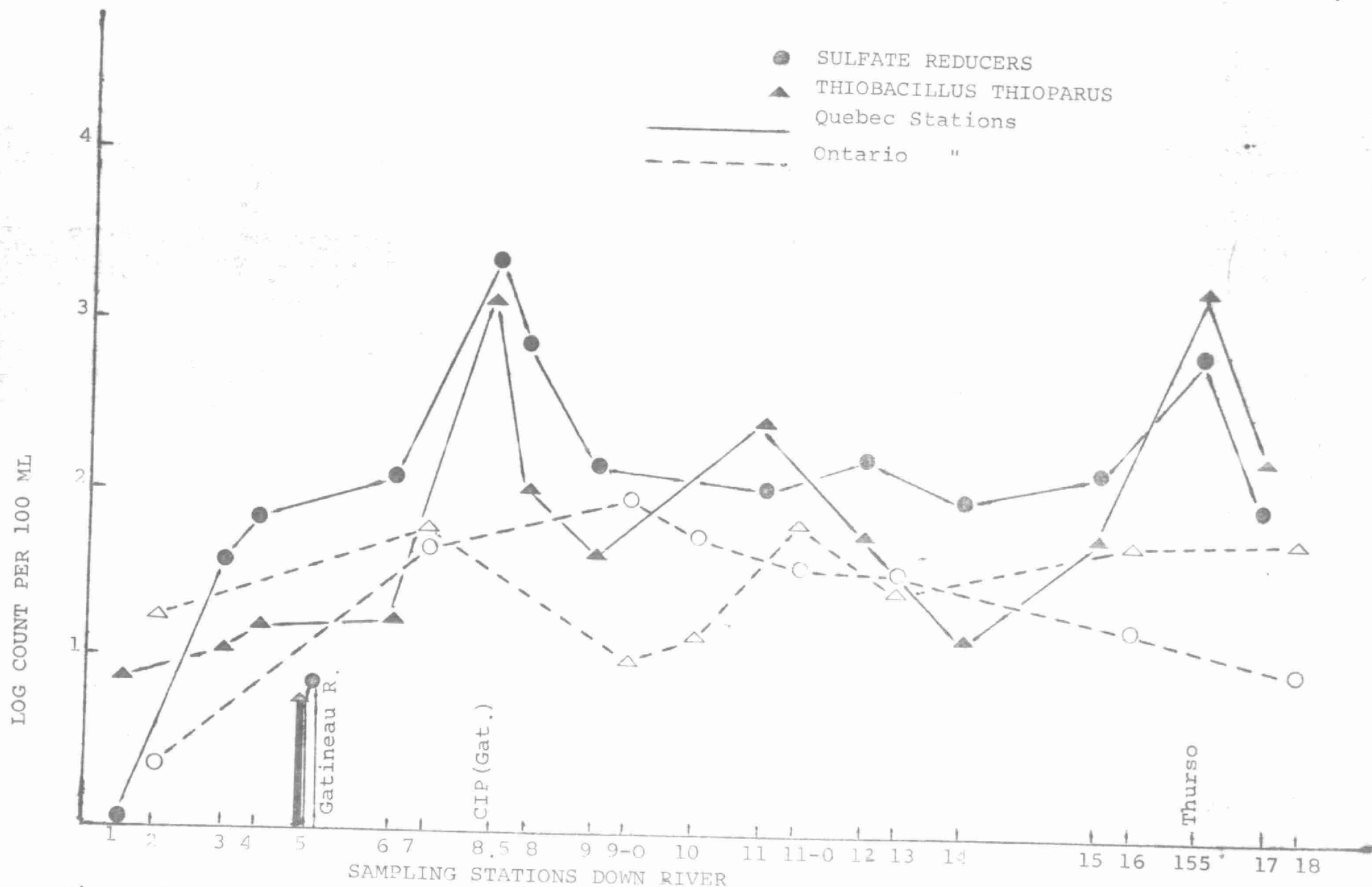


Figure 8- Mean distribution of sulfate-reducing and sulfur-oxidizing bacteria in surface-water of the Ottawa River at selected stations between Ottawa & Thurso during the summer of 1969.

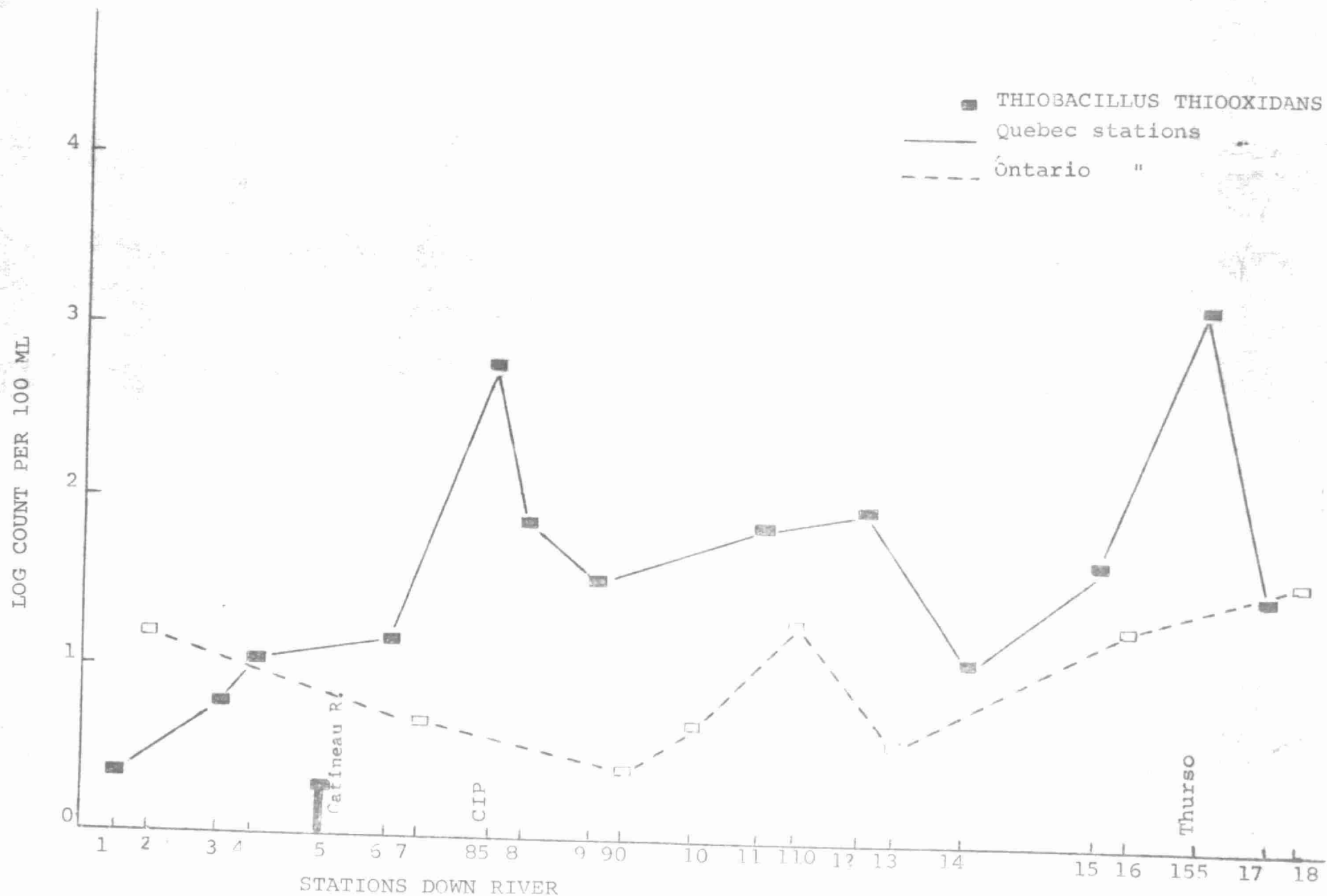


Figure 9- Counts of Thiobacillus thiooxidans, in surface water of the Ottawa R. at selected stations between Ottawa & Thurso (summer, 1969).

Counts of Thiobacillus thiooxidans (Fig. 9) in water closely paralleled those of T. thioparus (Fig. 8) at the sampling stations down river. Numbers of the sulfur-oxidizing bacteria reached maximum levels at Quebec stations 8.5, 11 and 15.5 and at the Ontario station, 11-0. Below Duck Island, mixing of water from the Quebec channel may have contributed to the increased population of sulfur-oxidizers in the water at 11-0. Data obtained in August, 1968 (Fig. 14) indicated a trend in population distribution of T. thiooxidans in the surface water similar to that observed in 1969.

Sulfate-reducing bacteria appeared in highest concentration in surface water near the mill outfalls at CIP (Gatineau) and Thurso, where substrate for growth was probably most abundant, and conditions more reducing than in reaches of the river where the mill effluent was more dilute. The increased number of sulfur-oxidizing bacteria at 11 and 11-0 may be due to growth of the microorganisms in water on reduced inorganic sulfur (e.g. sulfite, sulfide, thiosulfate) liberated from organic sulfur compounds in the effluent from CIP (Gatineau), or produced by the activities of sulfate-reducers in the sediment. The decomposition of organic sulfur compounds, e.g. alkyl sulfide, mercaptans and lignosulfonates by heterotrophic bacteria in water would release a reduced inorganic form of sulfur, available for metabolism by autotrophic thiobacteria. Bollen (1) reported on the oxidation of sulfur from lignin sulfonates in waste sulfite liquor by the thiobacilli.

Although no correlation could be drawn between dissolved oxygen concentration and bacterial counts, the maximum population of sulfur oxidizers occurred at station 11, near the Quebec shore below Duck Island and where a D.O. sag was observed (Fig. 11). The mean total plate count, sulfate-reducer and coliform counts did not reach a significant peak at station 11 (Figs. 8, 10) in surface water. The metabolism of sulfur oxidizers would consume dissolved oxygen, but to state that the D.O. sag at station 11 is entirely due to the activities of the sulfur bacteria would definitely be preclusive at this time. The relationship between D.O. and Microbial oxidizing activity may be more significant than the correlation with bacterial numbers, since it is the metabolic activities of the population of cells, not the actual numbers of cells present that influence the rate and quantity of O_2 consumed in water. Hence a more meaningful and informative approach to aquatic microbial ecology could be made by a more direct study of the biochemistry and physiology of microorganisms in water, rather than a mere enumeration of a small segment of the total bacterial population.

Mean total plate counts and total coliform counts for the summer were determined and plotted on the graph (Fig. 10). Total plate counts were approximately one order of magnitude greater than coliform counts at all stations, and a close parallel between the two parameters could be seen. The greatest disparity was observed

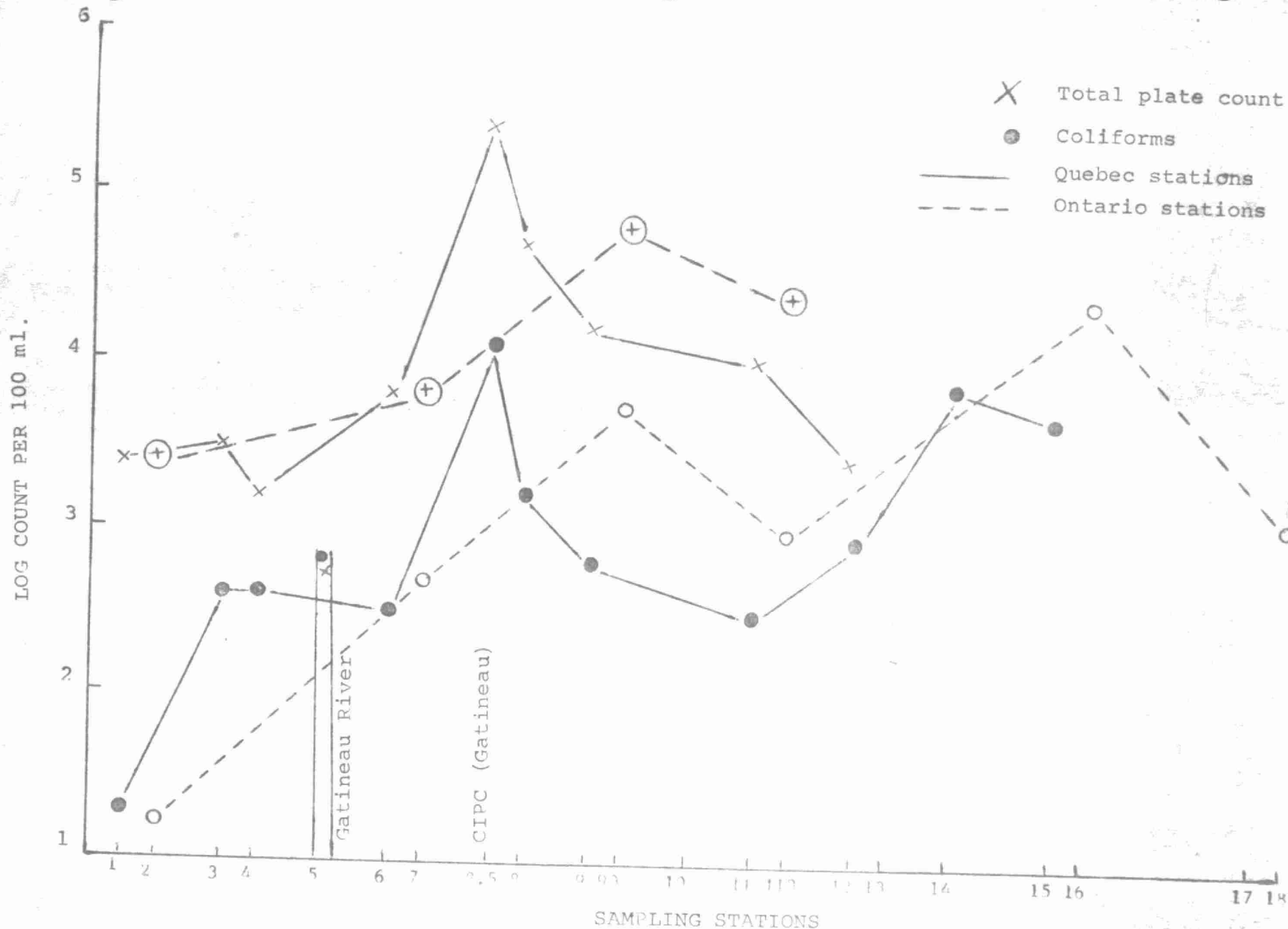


Figure 10 - Mean total plate count and coliform count in surface water at selected stations on Ottawa River for summer of 1969.

D.O. (p.p.m.) at 20 C.

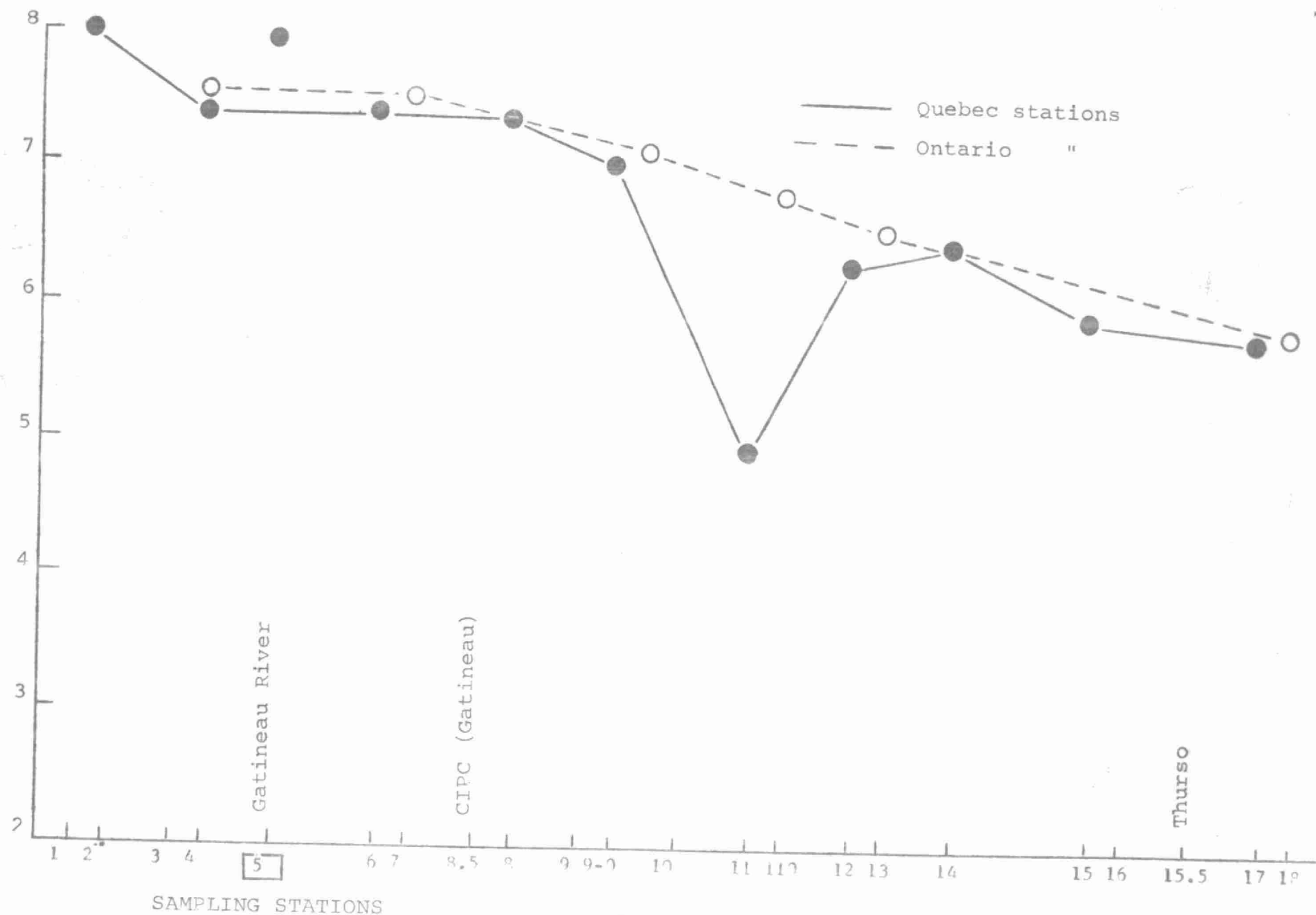


Figure 11 - Mean dissolved oxygen levels in surface water of Ottawa River between Ottawa & Thurso (summer, 1969).

at stations 1 and 2 on Lac Deschenes where a low coliform population of $20/100$ ml contrasted to a moderate total plate count of 2.5×10^3 per 100 ml. The coliform population increased to $4.0 \times 10^2/100$ ml at stations 3 and 4 in Hull-Ottawa and below Eddy (Hull), while no such increase appeared in total count at this point. Both coliforms and total plate count increased to 1.3×10^4 and 2.5×10^5 per 100 ml respectively near station 8.5, just below the outfall from CIP (Gatineau) on the north channel of Kettle Island. Between Quebec stations 8 and 12 from Kettle Island to above the mouth of the Lievre River, numbers of coliforms were relatively constant in the order of approximately $10^3/100$ ml, while total plate count decreased from $5.0 \times 10^4/100$ ml at station 8 to $2.5 \times 10^3/100$ ml at 12. No significant difference was observed between counts of either parameter on the Ontario or Quebec side of the river at 1 and 2 (Lac Deschenes) and 6 and 7 (Rockcliffe Park). In contrast to populations of sulfur bacteria, counts of coliforms and total plate counts were higher near the Ontario side than at Quebec stations at ranges 9 & 9-0, 11 & 11-0 and 15 & 16. High heterotrophic bacterial densities at these Ontario stations may be due to the influence of the Ottawa STP near Lower Duck Island. At the Quebec stations, there appeared to be a contribution of coliforms and saprophytic bacteria from the outfall of CIP (Gatineau) (8.5) with a subsequent reduction in numbers further down river due to die-off and/or dilution of bacterial cells.

The mean distribution of sulfate-reducing and sulfur-oxidizing bacteria in sediment samples from the Ottawa River between Ottawa and Thurso is shown in Figures 12 and 13. Sulfate-reducers were not detected ($< 100/g$) in the sediment samples from Lac Deschenes (stations 1 and 2) and from the Gatineau River (station 5). Peak populations of Desulfovibrio were found in bottom samples from Quebec stations 4 (below Eddy (Hull)), 8 (below CIP (Gatineau)), 14 (below mouth Lievre River) and at 17 (below Thurso mill) where numbers reached approximately 10^4 cells per gram sediment. Counts of sulfate-reducers in the sediment near the Ontario shore exceeded the count at the corresponding Quebec station across river only at station 11-0 below Lower Duck Island where numbers reached $3.6 \times 10^3/g$ sediment. This was also observed in August 1968 (Fig. 14), when peaks in the Desulfovibrio population occurred at the stations below Eddy (Hull) and CIP (Gatineau) on the Quebec side, and at Ontario station 11-0. The relatively high count of sulfate-reducing bacteria found in the sediment at station 11-0 may be due to the influence of wastes from the Ottawa STP or the effects of pollutants carried by currents from the Quebec channel to the Ontario side below Lower Duck Island. Since numbers of sulfate-reducers were lower in sediment at station 10 (also below the Ottawa STP) than at 11-0, it is most likely that the mixing of water from the Quebec side contributed to the high levels of sulfate-reducers at station 11-0.

SEDIMENT

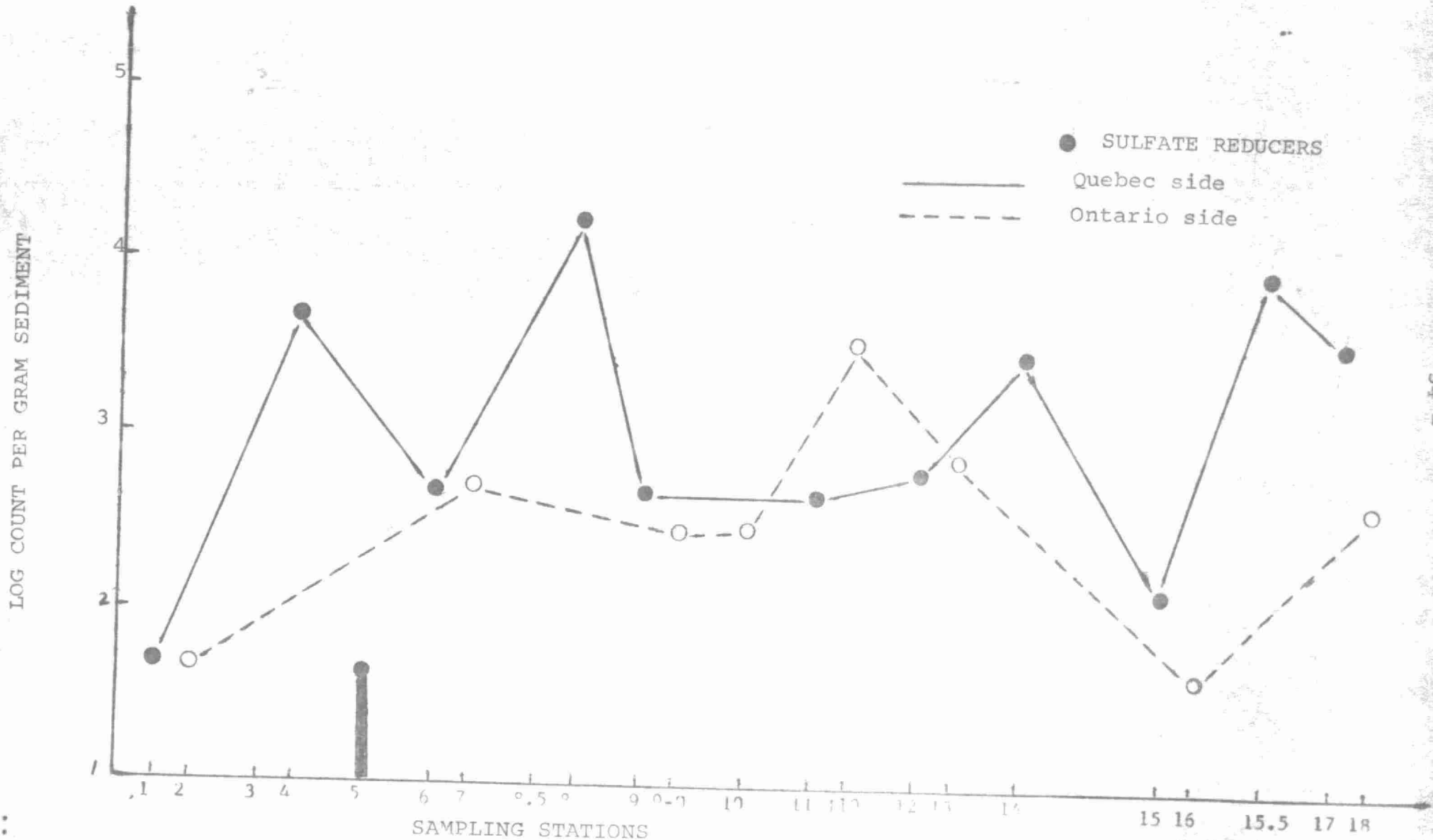


Figure 12 - Mean distribution of sulfate-reducing bacteria in sediments of the Ottawa River at selected stations between Ottawa & Thurso (summer, 1969).

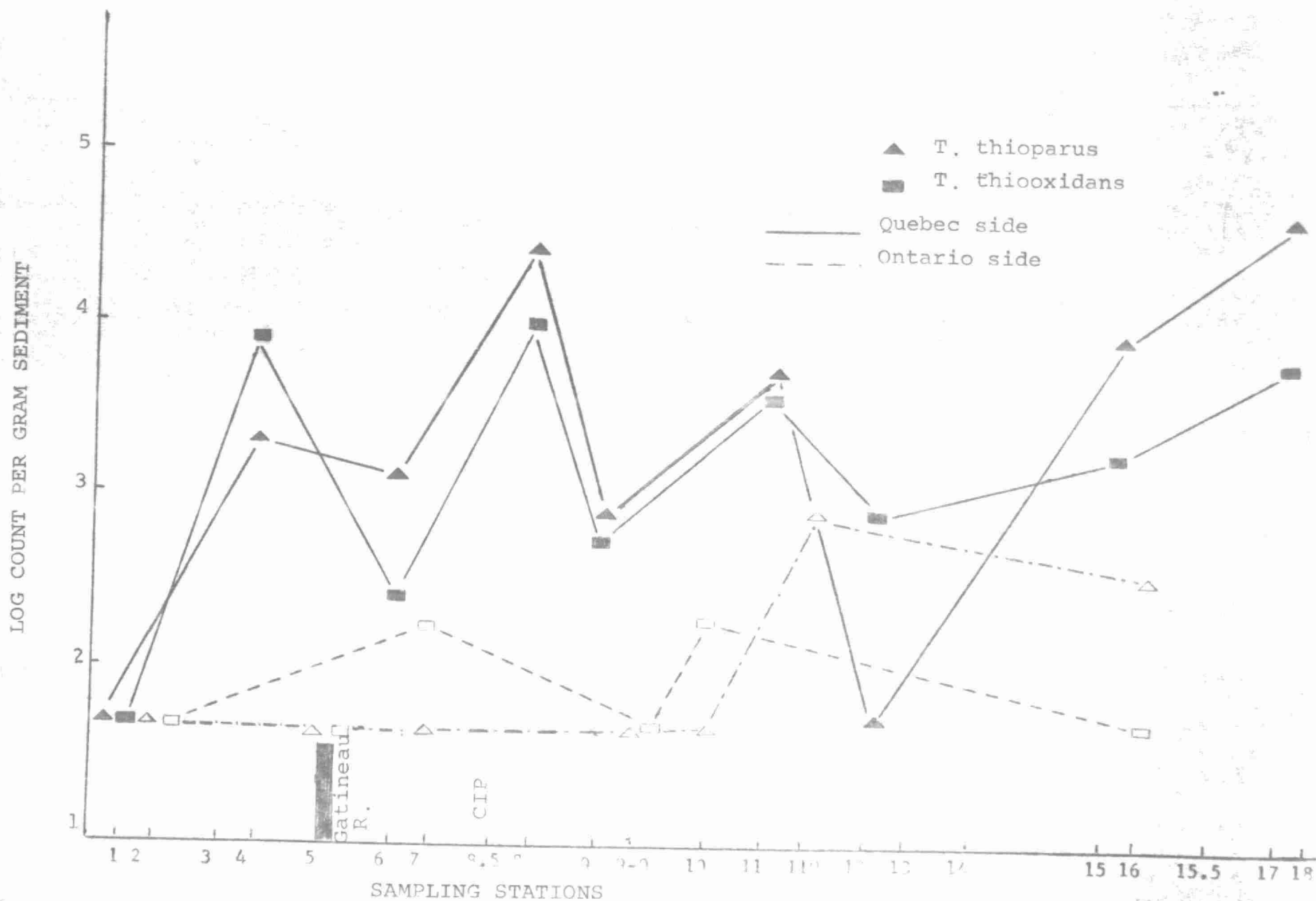


Figure 13 - Mean distribution of sulfur oxidizing bacteria in sediments of the Ottawa River at selected stations between Ottawa & Thurso (summer, 1969).

Since counts of sulfate-reducers in the river bottom were maximal immediately below the pulp and paper mills, Eddy (Hull), CIP (Gatineau) and Thurso, it may be concluded that components of paper mill wastes were contributing to favorable growth of these bacteria in the sediment.

It was observed (Fig. 13) that counts of Thiobacillus thioparus and T. thiooxidans in sediments paralleled one another closely at each station, although densities of T. thioparus were generally greater. It is possible that the two distinct species T. thioparus and T. thiooxidans may have been present in approximately equal numbers, the former growing at a neutral pH, the latter at acid pH's or a species T. intermedius, with broad pH requirements over the range 7.0 - 2.0 may have grown on both types of thiobacillus media. At stations near the Ontario shore of the river, the distribution of thiobacilli was in the order of 10^2 or less per gram sediment, while these were significantly greater at Quebec stations within the range, $10^2 - 10^4$ /g sediment. Thionic bacteria were not detected in Lac Deschenes or in Gatineau River mud samples (< 100 /g). Maximum numbers of sulfur oxidizing bacteria in the order of 10^4 /g appeared in the river sediment from stations 4, 8 and 15.5, below the three paper mills. A high count of 6.3×10^3 /g and 8.0×10^2 /g occurred at Quebec and Ontario stations respectively below Lower Duck Island. At 11-0, near the Ontario shore, transport of sulfur substrates by currents from the Quebec channel mixing with the Ontario waters may have contributed the high levels of sulfur oxidizers in the sediment.

In all likelihood, deposition of sulfur compounds serving as energy sources for the thiobacilli at stations below the mill outfalls on the Quebec side were responsible for the high levels of sulfur oxidizers observed at these stations.

Desrochers and Fredette (3) surveyed an area of the Ottawa River between l'Orignal and Chute-a-Blondeau for sulfate-reducing bacteria (Desulfovibrio). They observed a considerable increase in the number of Desulfovibrio below the mill outfalls from the pulp and paper mill at Hawkesbury, counts sometimes exceeding 10^6 /ml. The greatest numbers of sulfate-reducers were found in June, August and September. In May and November, the populations were less than 10^3 per ml. They found a direct correlation between numbers of Desulfovibrio and B.O.D. and found highest populations in water where the D.O. was lowest. These workers concluded that the limiting factor in the development of Desulfovibrio in the Ottawa River was the redox potential and not the organic or inorganic nutrient supply.

The section of the Ottawa River between l'Orignal and Carillon surveyed by OWRC in 1969 is depicted in the maps (Figures 6 and 7 on pages 21 and 22). The mean distribution of sulfate-reducing bacteria in surface water at stations in the Hawkesbury area is shown in Fig. 15. Above Hawkesbury, mean populations of sulfate-reducing bacteria in the water at stations 19 and 20 was recorded as $< 2/100$ ml near the Ontario side compared to $20/100$ ml $1/4$ way from the Quebec shore. At the Quebec stations 21 at Grenville and 23 below Perley Bridge,

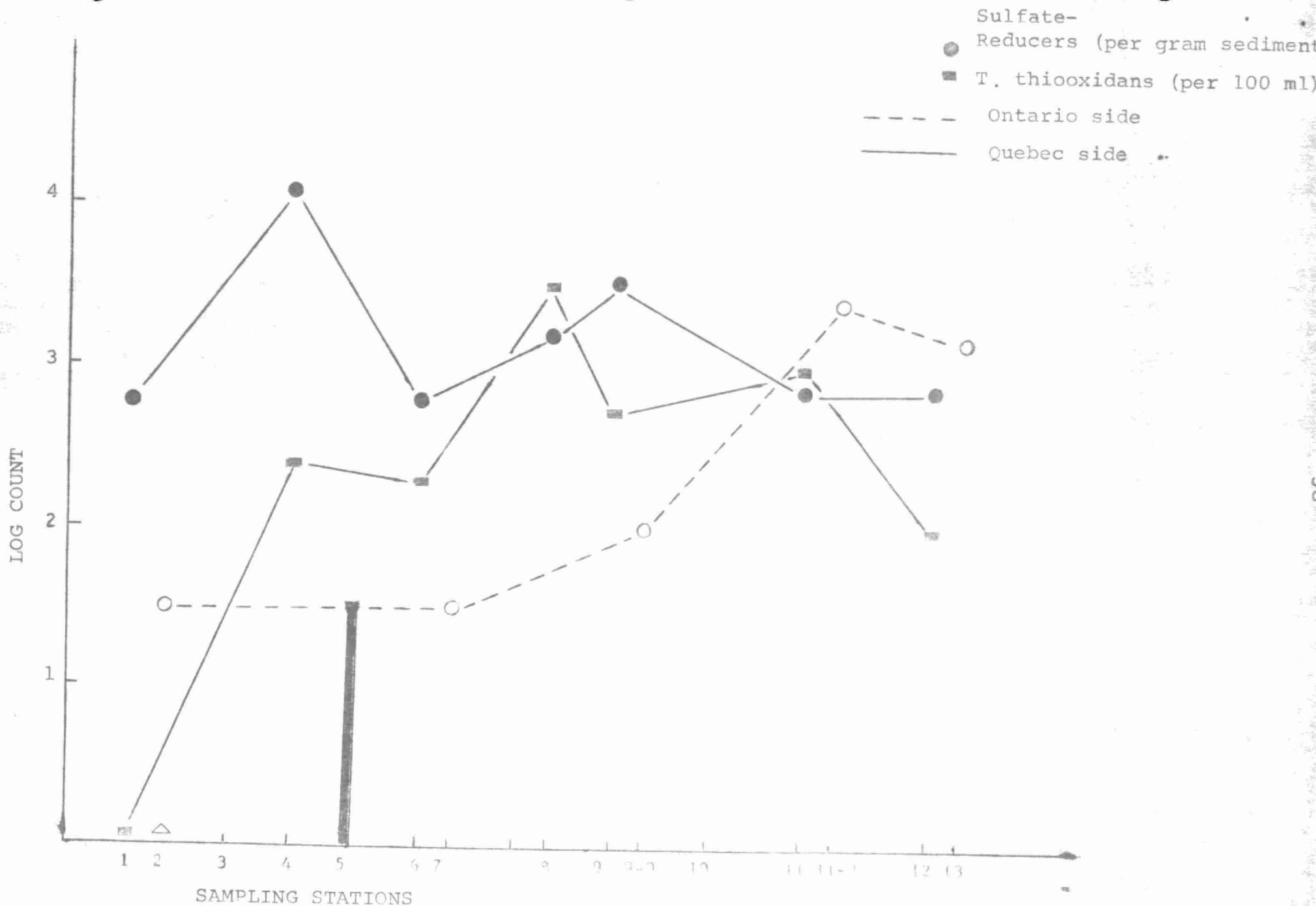


Figure 14

Counts of sulfate-reducers in sediment and T. thiooxidans in surface water samples taken in August, 1968 from the Ottawa River near Hull-Ottawa region.

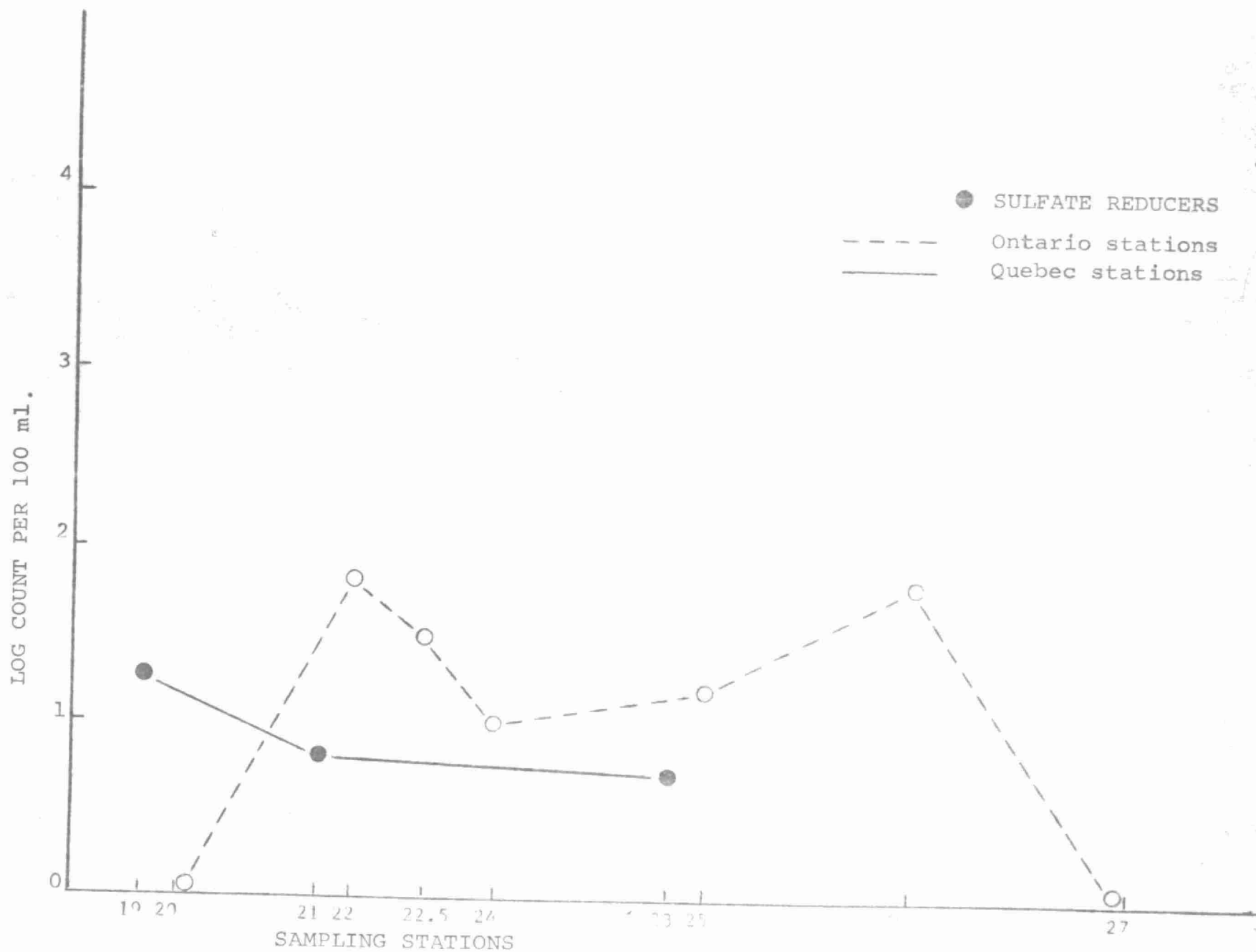


Figure 15 - Mean distribution of sulfate-reducing bacteria in surface water at sampling stations on Ottawa River (l'Original-Carillon) summer, 1969.

counts were below 10 cells per 100 ml. At the CIP mill at Hawkesbury between the Ontario shore and Hamilton Island, numbers reached 62/100 ml. At station 22 and at the effluent point discharge (22.5) a mean count of 31/100 ml was recorded. At Ontario stations 24, 25 and 26, counts fell into the range between 10 - 70 per 100 ml and declined to < 2/100 ml at 27 below Chute-a-Blondeau.

Relatively low populations of sulfur oxidizing bacteria (< 10/100 ml) were detected at stations 19 and 20 above Hawkesbury (Fig. 16). At the CIP mill (Hawkesbury), numbers of thiobacilli showed a substantial increase to approximately 2.0×10^2 /100 ml at stations 22 and 22.5. Counts were much lower at station 24, in the East channel between Hamilton Island and Perley Bridge (approximately 10/100 ml) while at 25, below Perley Bridge in the South channel along the Ontario shore, counts increased to the order of 10^3 /100 ml. Since populations of thiobacilli and sulfate-reducers were significantly higher at station 25 than at 24, it is probable that most of the Hawkesbury mill waste water was following the Ontario shore and detected at 25 rather than moving northward along the east side of Hamilton Island to the mid-river channel at Perley Bridge.

Although T. thiooxidans was not detected at sampling stations near the Quebec shore, low populations of T. thioparus (10 - 60/100 ml) were determined in water at these sites.

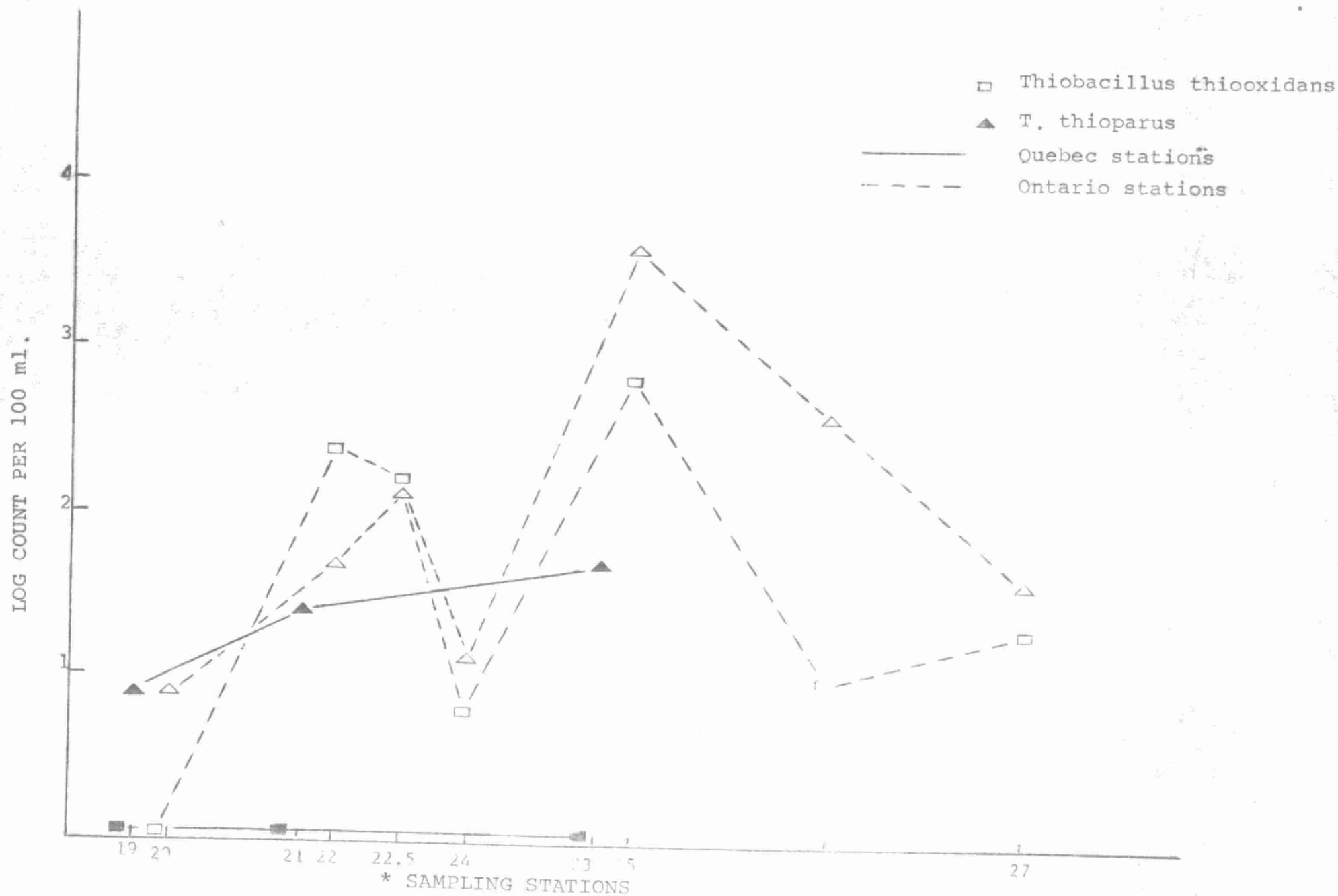


Figure 16 - Mean distribution of Thiobacillus thioparus and T. thiooxidans in surface water at selected stations of the Ottawa River (l'Original-Carillon) during summer, 1969.

Nitrifying bacteria were not detected in the water samples taken in June from this reach of the river ($< 3/100$ ml). However in August, numbers of Nitrosomonas, the ammonia-oxidizing bacteria varied from $< 3/100$ ml in surface water above and below Hawkesbury at stations 19, 20 and 27 to a maximum of 460/100 ml at station 25 below CIP (Hawkesbury) (Appendix Table III).

The mean distribution of sulfate-reducing and sulfur oxidizing bacteria in the sediment at various stations of the Ottawa River near Hawkesbury for the summer of 1969 is depicted in Figures 17 and 18. Above Hawkesbury, counts of sulfate reducers were in the order of 10^2 per gram sediment at both Ontario and Quebec stations and remained near this level at Grenville (station 21) and below Perley Bridge (station 23) on the Quebec side. In contrast, a significant increase in Desulfovibrio was observed at CIP (Hawkesbury) where numbers in the river bottom reached approximately 10^4 per gram at stations 22 and 22.5. The population remained high at $1.5 \times 10^4/g$ below the mill at station 25, east of Perley Bridge near Ontario side, although no sulfate-reducers were detected in the sediment at station 24 below the mill, off the east side of Hamilton Island. The population of sulfate reducers in the sediment declined slightly to the order of $10^3/g$ at stations 26 and 27 below Hawkesbury.

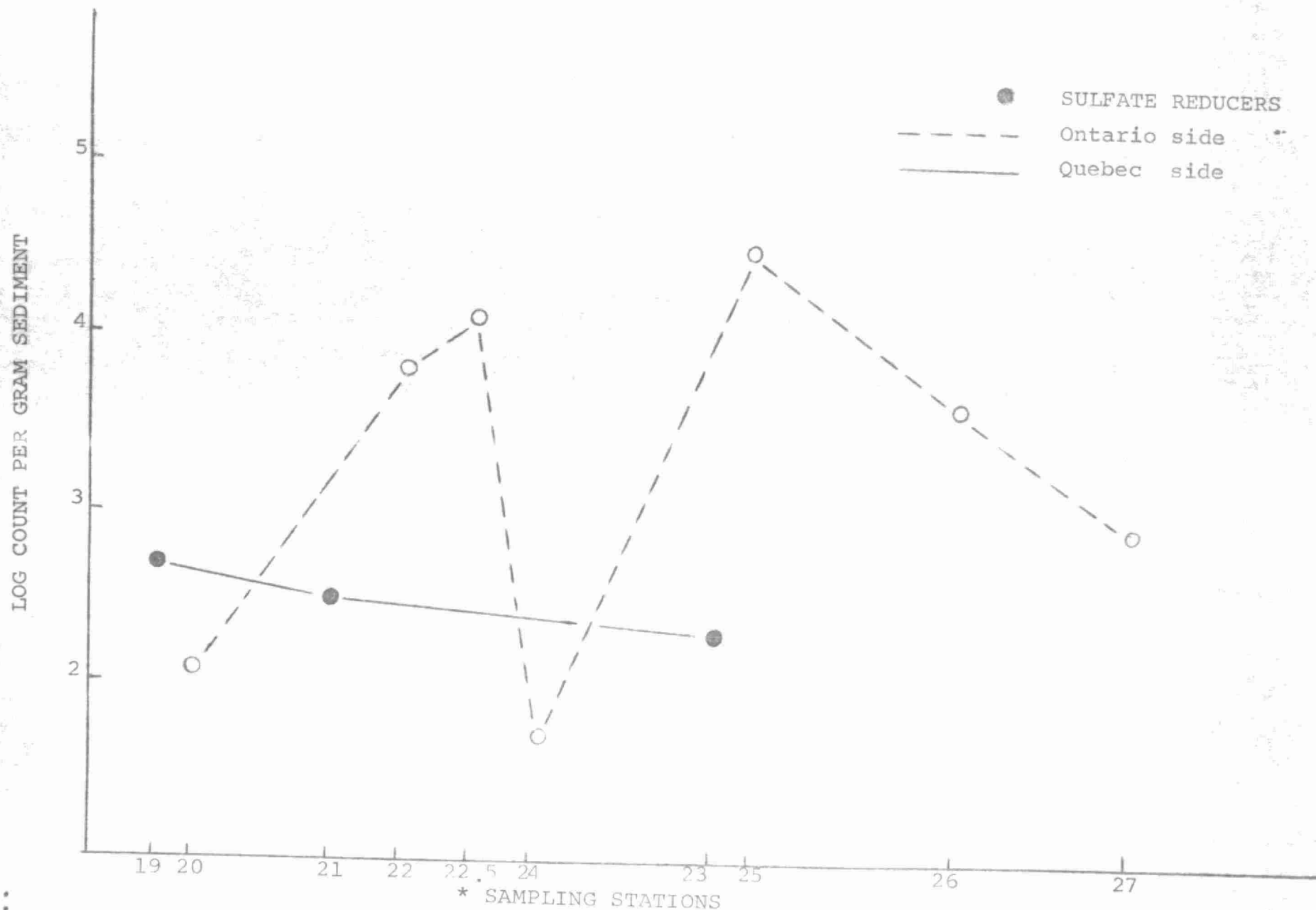


Figure 17 - Mean distribution of sulfate-reducing bacteria in sediment samples from selected stations of the Ottawa River from l'Original-Carillon (summer, 1969).

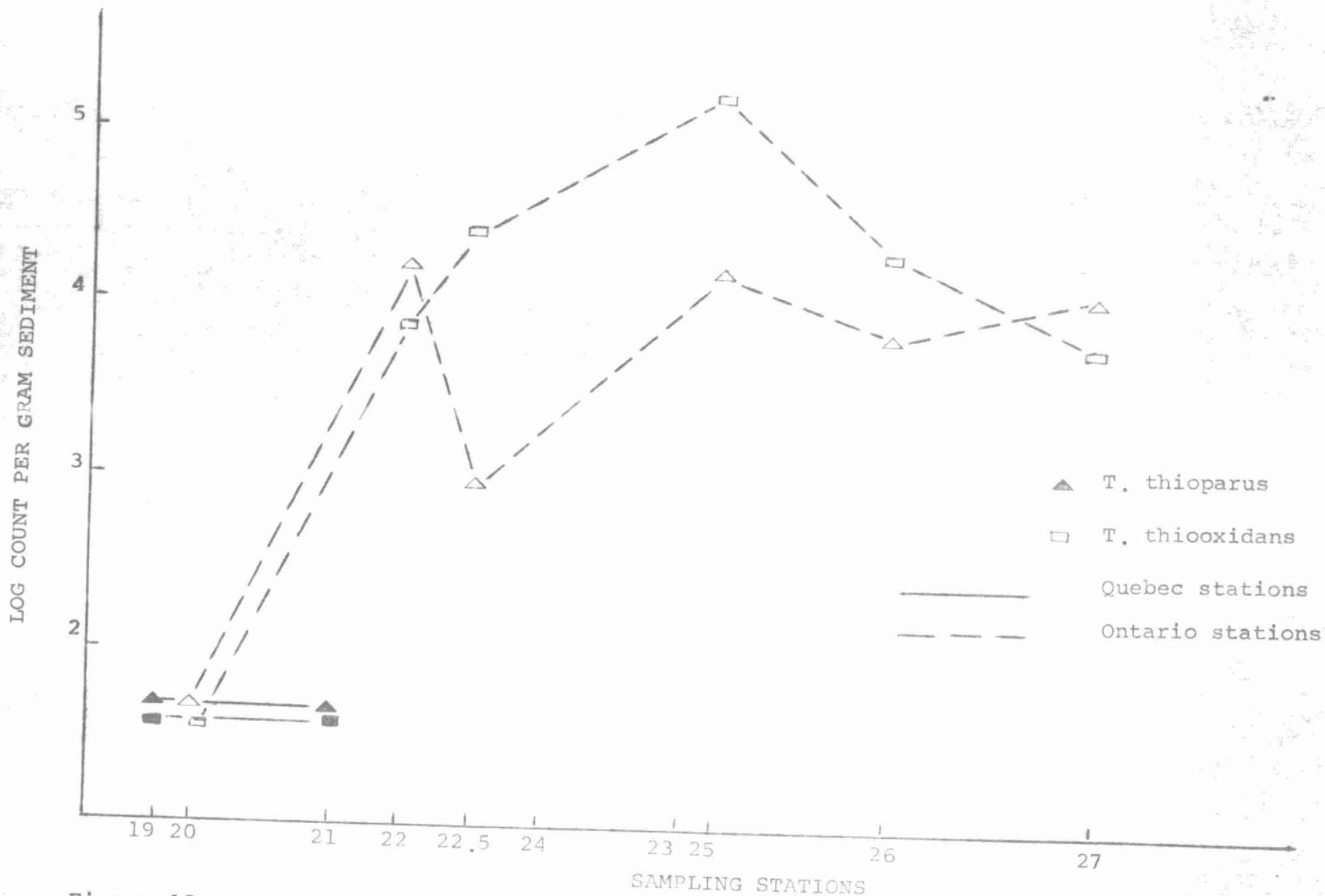


Figure 18 - Mean distribution of sulfur oxidizing bacteria in sediment samples from selected stations of the Ottawa River from l'Orignal-Carillin (summer, 1969).

Upstream of Hawkesbury, no sulfur oxidizing bacteria were found in the sediment (Fig. 18). Below CIP (Hawkesbury), the count increased to $10^3 - 10^4$ /g sediment on the Ontario side, while across the river on the Quebec side at Grenville, none were detected (< 100 /g). A maximum count of $10^4 - 10^5$ Thiobacillus cells per gram was recorded at station 25.

These results would strongly suggest that a greater portion of the pollution load from the Hawkesbury mill discharge was reaching station 25 than 24, and that the sulfate-reducing and sulfur oxidizing bacteria in the sediment were responding to input of sulfur substrates by the mill effluents. Because counts of sulfur bacteria in the sediment were still significantly high at stations 26 and 27, near Chute-a-Blondeau, it is postulated that sufficient concentrations of sulfur compounds were being cycled in the sediment to maintain a strong population, at this mileage range below Hawkesbury.

It is apparent from Figure 19 that the coliform count did not significantly increase below Hawkesbury as did counts of sulfur bacteria in the summer 1969. No significant difference was observed between the total coliform population near the Quebec and Ontario shores of the river, with counts all within the range $10^2 - 10^3$ /100 ml. Mean total plate counts increased significantly from 1.5×10^3 /100 ml at Ontario stations above

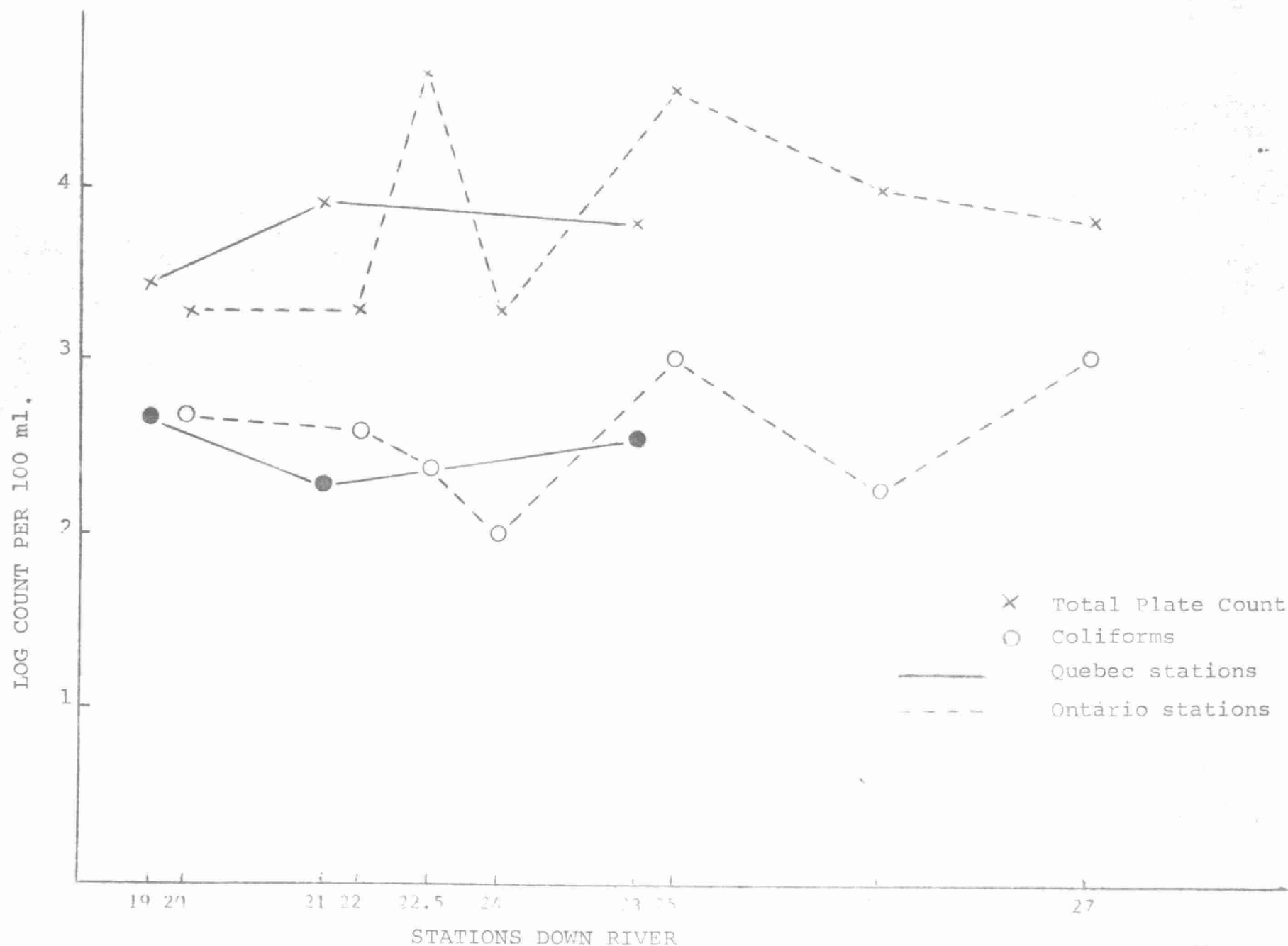


Figure 19 - Mean coliform and total plate counts in surface water of the Ottawa River (l'Original-Carillon) summer, 1969.

Hawkesbury to approximately $7.0 \times 10^4/100$ ml at stations 22.5 and 25, near the Ontario shore below CIP (Hawkesbury). However, these counts did not vary greatly from those obtained at Quebec stations 21 and 23 of nearly $10^4/100$ ml. It was thus obvious that sulfate-reducing and sulfur-oxidizing bacteria were more sensitive parameters in indicating the outfall from CIP (Hawkesbury) than coliforms and total plate count, since increases in populations of the former groups of bacteria were more marked at stations below CIP (Hawkesbury) on the Ontario side than fluctuations in counts of the heterotrophic types.

The mean D.O. content of the water for the summer at stations above and below Hawkesbury, near the Ontario and Quebec shores remained at a relatively low value of approximately 5 ppm (Fig. 20). No correlation was apparent between D.O. concentration and bacterial counts in the water or sediment.

The 5-day BOD values in surface water, above and below Hawkesbury averaged 2 - 3 ppm for the summer. This fact was also apparent for other locations on the river, e.g. CIP (Gatineau) where no excessive increase in BOD was noted in surface water downstream from the mill effluent (Data available from OWRC Water Quality Surveys Monitoring Program). This would lead one to speculate that components of the paper mill wastes were in a fairly stable state and relatively resistant to rapid oxidation by the time the waste waters reached the stations below the mill outfalls.

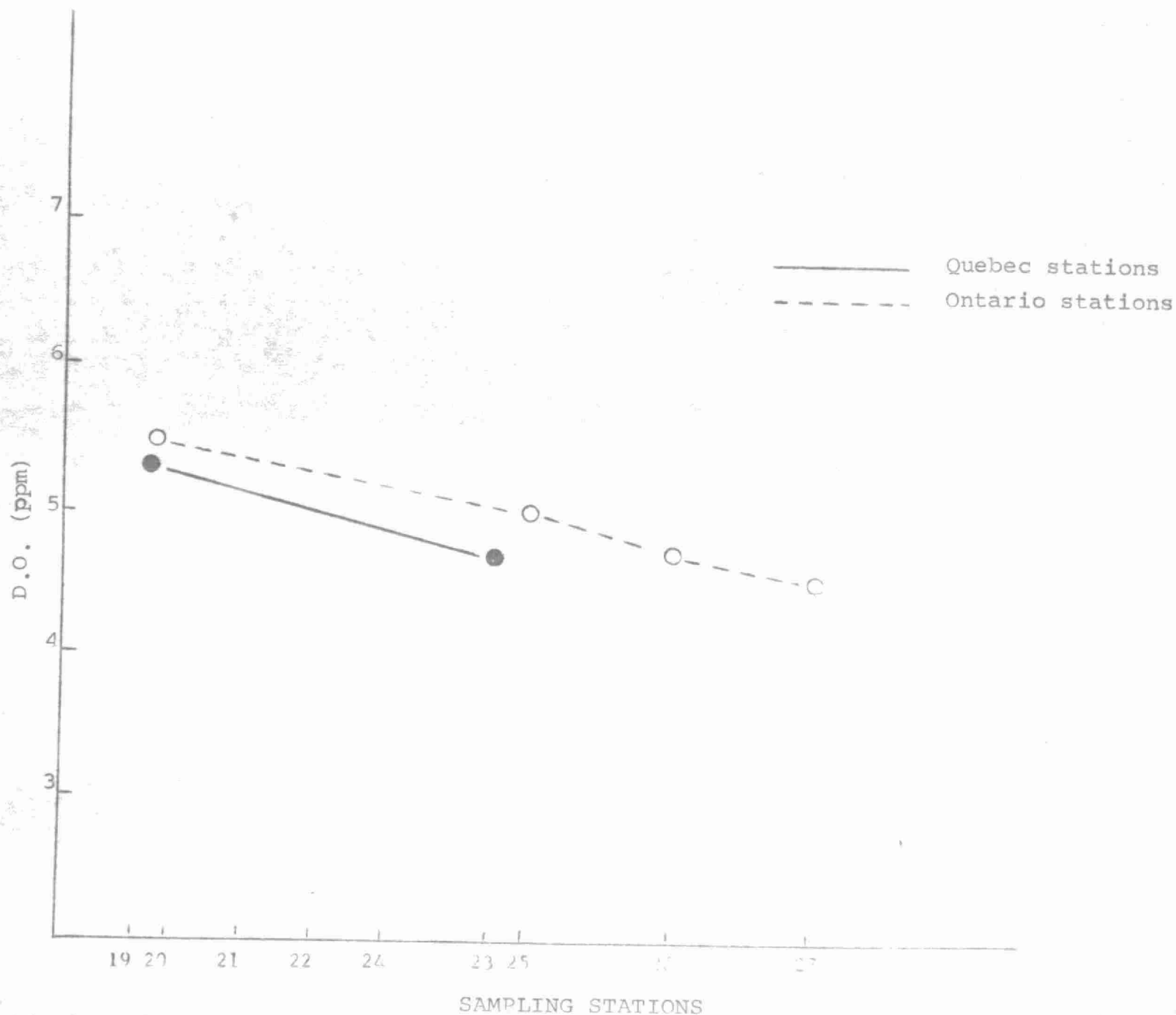


Figure 20 - Mean dissolved oxygen levels in surface water of Ottawa River (l'Original-Carillon) during summer of 1969.

Lignosulfonates which constitute the bulk of waste sulfite liquor, and cellulose, are refractory to biodegradation, not yielding to ready oxidation by the aquatic microflora. D.O. sags or depressed D.O. conditions as evident in the Hawkesbury region may be due to the oxidation of sulfur from lignosulfonates discharged many miles upriver, the microflora requiring a long adaptation period to synthesize the requisite enzymes capable of splitting the sulfo-group from the lignosulfonate molecule. Thiobacilli then could consume dissolved oxygen in the process of oxidizing the inorganic sulfite liberated to sulfate. The possibility that other resistant organic compounds in paper mill wastes, e.g. cellulose, may be implicated in D.O. sags should not be overlooked also. The production of simple reduced organic or inorganic substances, e.g. CH_4 , H_2S , from the sludge mats on the river bottom could also be involved in creating an oxygen demand in surface water.

Besides the ecological factors affecting the population and activities of the aquatic bacteria, one must also take into account other environmental parameters, e.g. mixing of waters from different shorelines, river flow characteristics, surface reaeration, benthic demand, algal photosynthesis, water temperature, characteristics of paper mill waste (BOD - readily degradable and refractory materials), and local sources of pollutant discharge, when considering the complex of interacting forces influencing the O_2 balance of a river. All those factors contribute to the

intricacies of the D.O. - BOD system and there are no justifiable grounds for assuming that bacterial counts, i.e. population densities ought to be correlated with D.O. or BOD. This has been substantiated from the data and results of this survey.

Organic compounds derived from wood pulping are diverse in nature and are subject to microbiological action in water. Readily fermentable wood sugars (hexoses, pentoses) of waste sulfite liquor stimulate the growth and activities of the natural microflora in water at the time of discharge. The immediate capacity for growth of the microflora may be limited by one or more factors, other than food supply, e.g. availability of D.O. for aerobic organisms, accessory nutrients (N,P).

A study of the influences of pulp and paper mill wastes on sulfate-reducing and autotrophic sulfur-oxidizing bacteria in sediments and surface water of the Ottawa River demonstrated that enrichment of the environment with these bacteria occurred predominantly in zones of the river below mill discharge. However, much still remains to be learned about the microbiological reactions of waste liquors from pulp and paper mills in natural waters.

A bacteriological survey of the Ottawa River was conducted during the summer of 1969 and concentrated on sections of the river where water quality was impaired by wastes from pulp and paper industries. Counts of sulfate-reducing and sulfur-oxidizing bacteria in sediment and surface water were significantly greater in zones below than in areas above the outfalls from paper mills. These parameters appeared to be relatively more sensitive than coliform counts or total plate counts in indicating reaches of the river, in proximity to the mill outfalls where water was most heavily polluted by these industrial wastes. Sulfur substrates which are normally lacking in natural, unpolluted river water, but are found in significantly high concentrations in waste sulfite and kraft liquors apparently contributed to the increased populations of sulfur bacteria found in zones below mill outfalls.

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APPENDIX

TABLE I - Bacterial counts in surface water of the Ottawa River
below Temiskaming during July 21-26 (1969).

Count per 100 ml. surface water			
Station	Sulfate Reducers	T. thioparus	Plate count
1	20	< 3	9,500
2	140	9	
A-2	7,000	1,500	
A-3	460	2,100	38,000
A-4	-	-	42,000
A-5	45	43	8,300
B-6	150	1,800	-
B-7	-	-	20,000
B-8	110	600	-
C-10	-	-	96,000
D-12	350	430	-
D-13	-	-	63,000
D-14	410	230	-
E-15	-	-	75,000
F-19	-	-	47,000
F-20	80	230	-
G-21	-	-	50,000
H-24	-	-	17,000
I-27	-	-	17,000
J-30	-	-	37,000
K-33	-	-	38,000
L-37	< 4	< 3	21,000
M-40	8	40	21,000
M-41	-	< 3	-
N-43	10	< 3	-
MR-1	< 4	< 3	-
U-64	15	< 3	-
Z-79	10	< 3	-

TABLE II - Bacterial counts in sediments at selected stations
of the Ottawa River (Temiskaming-Mattawa - July 1969).

Station	COUNT PER GRAM SEDIMENT	
	Sulfate Reducers	T. thioarum
A-3	22,000	1,600
A-5	3,100	1,700
B-6	8,700	105
B-8	2,500	220
C-9	2,900	750
D-12	40,000	1,300
D-14	7,700	3,300
F-20	320	< 50
L-37	590	< 50
M-40	< 100	< 50
MR-1	< 100	< 50
N-43	< 100	< 50
U-64	280	< 50
Z-79	2,000	< 50

TABLE III - Bacterial counts in Ottawa River water (Ottawa-Carillon).

Count per 100 ml.

Station	Sulfate Reducers			Thiobacillus Thioparus			T. Thiooxidans			Log Plate Count (mean for summer)	Nitrifiers	
	June 17	Aug.	Sept. 12	June	Aug.	Sept.	June	Aug.	Sept.		June	Aug.
1	<2	<2	<2	<3	93	9	<3	15	<3	3.37		
2	15	<2	<2	<3	23	240	<3	43	240	3.36		
3	18	-	70	<3	93	15	<3	9	4	3.49		
4	24	-	140	<3	43	93	7	7	43	3.17		
5	50	-	<2	<3	210	<3	<3	7	<3	2.81		
6	-	-	470	-	240	23	-	9	23	3.79		
7	42	-	60	<3	4,600	43	<3	4	23	3.00		
8	2,300	-	230	21	1,500	43	23	240	93	4.72		
8.5	6,000	-	920	4,600	21,000	43	2,400	460	210	5.35		
9	160	-	150	11	460	23	9	240	23	4.22		
9-0	30	-	330	<3	1,100	<3	<3	15	<3	4.83		
10	60	-	80	<3	1,100	4	9	15	<3	2.92		
11	150	-	100	150	460	460	23	150	150	3.97		
11-0	-	-	40	-	240	43	-	9	43	4.38		
12	140	200	350	14	-	240	43	-	240	3.26		
13	46	250	4	<3	-	1,100	<3	-	15			
14	40	70	450	15	-	-	21	-	9			
15	175	820	20	21	-	210	23	-	93			
15.5	75,000	260	2,200	4,600	-	1,500	1,500	-	4,600			
16	68	160	<2	7	-	46	20	-	23	-		
17	190	200	18	9	-	460	43	-	23	-		
18	10	15	6	<3	-	4,600	4	-	23	-		
19	80	100	<2	<3	<3	46	<3	<3	<3	3.44		
20	<2	<2	<2	<3	4	100	<3	<3	<3	3.32	<3	4
21	10	<2	22	4	<3	460	<3	<3	<3	3.89	<3	-

TABLE III - continued

Count per 100 ml.

Station	Sulfate Reducers			Thiobacillus Thioparus			T. Thiocoxidans			Log Plate Count (mean for summer)	Nitrifiers	
	June 17	Aug.	Sept. 12	June	Aug.	Sept.	June	Aug.	Sept.		June	Aug.
22	1,600	<2	76	-	<3	24,000	65,000	11	150	3.29	<3	21
22.5	<2	75	400	240	94	110	150	43	1,100	4.69	<3	<3
23	20	10	<2	7	14	460	<3	<3	<3	3.75	<3	-
24	26	2	40	<3	<3	460	<3	9	23	3.35	<3	-
25	40	<2	88	1,600	4,600	11,000	1,100	2,400	93	4.58	<3	460
26	76	400	10	4	15	46,000	<3	14	43		<3	-
27	<2	<2	<2	9	23	56	9	93	15		-	<3

TABLE IV - Bacterial counts in sediment samples from Ottawa River
(Ottawa-Carillon)

Count per Gram Sediment

Station	Sulfate Reducers			Thiobacillus Thioparus			T. Thiooxidans		
	June 17	Aug.	Sept. 25	June	Aug.	Sept.	June	Aug.	Sept.
1	<100	-	<100	<100	-	-	<100	-	-
2	<100	-	<100	<100	-	-	<100	-	-
4	7,800	-	3,800	2,200	-	-	8,800	-	-
5	<100	-	<100	<100	-	-	<100	-	-
6	<100	1,100	1,500	2,000	900	-	<100	700	-
7	<100	720	3,300	<100	<100	-	<100	400	-
8	26,000	15,000	24,000	13,000	51,000	-	6,400	23,000	-
9	500	<100	4,000	1,200	520	-	700	520	-
9-0	<100	360	900	<100	<100	-	<100	<100	-
10	<100	890	400	<100	<100	-	<100	480	-
11	650	<100	2,000	25,000	1,200	-	6,000	2,700	-
11-0	-	6,000	1,500	-	<100	-	-	<100	-
12	800	-	700	<100	-	-	800	-	-
13	-	-	2,100	-	-	-	-	-	-
14	2,000	-	7,000	-	-	-	-	-	-
15	<100	-	250	9,300	-	-	1,900	-	-
15.5	-	-	13,000	-	-	-	-	-	-
16	<100	-	<100	400	-	-	<100	-	-
17	1,400	-	13,000	50,000	-	-	7,300	-	-
18	-	-	500	-	-	-	-	-	-
19	<100	260	4,600	<100	-	850	<100	-	-
20	200	<100	<100	<100	-	-	<100	-	-

TABLE IV - continued

Count per Gram Sediment

Station	Sulfate Reducers			Thiobacillus Thioparus			T. Thiooxidans		
	June 17	Aug.	Sept. 25	June	Aug.	Sept.	June	Aug.	Sept.
21	<100	-	1,200	<100	-	-	<100		
22	3,800	3,100	27,000	16,000	-	9,200	7,200		
22.5	4,500	-	40,000	900	-	-	250,000		
23	-	480	<100	-	-	-	-		
24	-	-	<100	-	-	-	-		
25	22,000	21,000	49,000	14,000	-	150,000	200,000		
26	600	9,200	9,000	7,500	-	-	18,000		
27	400	<100	10,000	12,000	-	43,000	6,800		

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